University of Alberta

Lateral and Vertical Movement of Water, Nutrients, Fecal Coliforms, and Somatic Coliphages in Soils Dosed with Wastewater by Point and Drip Application Methods

by

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ABSTRACT

Lateral and Vertical Movement of Water, Nutrients, Fecal Coliforms, and Somatic Coliphages in Soils Dosed with Wastewater by Point and Drip Application Methods

Point and drip application methods are being used to disperse sewage effluent. The objective was to compare the lateral and vertical movement of water, NH_4-N, NO_3-N, Kelowna-P, fecal coliforms (FC) and somatic coliphages (SC) in packed soil columns and in a field soil. Soil columns were dosed (once/day) for 60 and 90 days with diluted primary effluent (DPE) from Goldbar wastewater treatment plant. The point method created transient saturated flow but, the drip method created transient unsaturated flow. FC only moved to a depth of 75 cm over 90 days but SC and nutrients were detected in soil leachates. DPE, with Brilliant Blue dye, was applied to field microplots (once/day) for 15 days. Wastewater was more evenly distributed in soil by drip versus point method. This also attenuated both organisms. The drip method provided a more effective treatment of wastewater than the point method. These methods need further field testing.

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Chapter 1

Research Rational and Thesis Overview

1.1 Introduction

An onsite sewage treatment system is any system that removes contaminants from wastewater at or near the point of generation with the dispersal of fluid nearby (Figure 1.1). Such systems can serve a single-family residence, a restaurant, an office building or a major resort (EPA, 2004). There is a wide range of private, onsite, soil-based wastewater sewage treatment systems in Alberta. These range from septic tanks with subsurface dispersal field; to septic tanks with mounds, to aerobic treatment units with subsurface or at-grade dispersal to advanced treatment plants with at-grade dispersal. Approximately 37.10 billion liters (9.8 billion gallons) of sewage are treated and disposed annually in Alberta by using onsite private sewage systems. According to industry representatives, over 535,000 people are using 200,000 to 250,000 private sewage treatment systems and about 7,000 new sewage systems are installed annually in Alberta (Durnie, 2002).

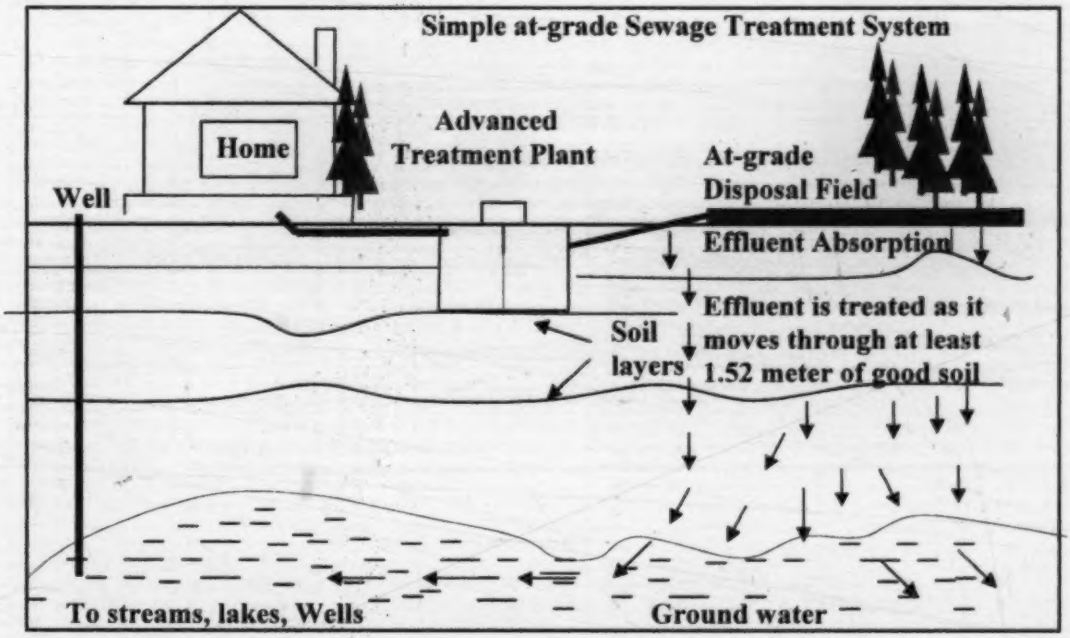

Figure 1.1. Diagram of a simple private, onsite, at-grade sewage treatment system.

In order to protect public health and water quality, Federal, Provincial and Local governments of Canada have established regulations for the application of treated sewage effluent to soil. The goal of the soil component is to renovate the wastewater and return it to the environment. Several factors such as depth of seasonal water table, presence of restrictive soil layers, wastewater permeability rates, horizontal setback distances to drinking water wells, presence of water bodies and wet lands, and other physical features are considered in selecting and locating appropriate onsite wastewater treatment systems. The ability of the soil to treat wastewater and the longevity of the system depends on the initial design of system, method and application rates of the wastewater, and potential of the soil to purify the wastewater.

1.2 Wastewater Composition and Its Application to Soil

The purpose of a septic tank is to separate solids from the liquid wastewater and to enhance the breakdown of contaminants by naturally available microorganisms especially by the bacteria. In a septic tank, solids sink, the liquid layer is mostly wastewater and the scum layer floats on the top. The septic tank functions as both a quiescent zone where solids settle out of suspension and as an anaerobic digester. The digestion process is quite efficient (decreases BOD_5, TSS, nutrient concentration, and microorganisms) and reaches maximum efficiency during the warmer times of the year. The solids separating ability of a septic tank is higher in the colder periods due to less gas generation and occurrence of resuspension of particulates (Loomis, 1996). The solid sludge in the tank is removed by septic tank pumpers every 1 to 5 years depending on the size of the tank and the number of people using it.

The clarified liquid from the septic tank is moved to the soil absorption area by gravity flow or pumped under pressure. The effluent is spread over the soil absoption area through perforated laterals spread over the absorption area. The laterals may be pressure distribution laterals or larger gravity flow laterals. As wastewater infiltrates into and percolates through the soil, fecal coliforms get adsorbed to the soil particles and the process of inactivating pathogens is initiated. Viruses travel a greater distance with water compared to bacteria due to their smaller size (Gantzer et al., 2001).

Sewage effluents can contain suspended solids (SS), a wide variety of decomposable organic compounds which create a biochemical oxygen demand (BOD_5), resistant organic compounds, nutrients such as ammonium, nitrate and phosphate, antibiotics, heavy metals and pathogens (helminthes, protozoa, bacteria and viruses) (Reddy et al., 1981; Loomis 1996; Carrol et al., 2006). The ability of the soil to treat these contaminants depends upon the soil physical, chemical and biological properties, environmental conditions present in the soil, and application rate of the wastewater (Carrol et al., 2006). In addition to these factors, the chemical composition of the effluent itself is the single most important non-soil factor which governs the extent of effluent treatment in soil (Loomis 1996).

1.3 Soil Physical Properties and Wastewater Movement

Soil moisture, texture and structure have profound influence on the quality of wastewater treatment. Hagedorn et al. (1978) found that fecal bacteria moved faster in coarser textured soil than in the fine textured soil. In coarse textured soils, especially in sand and gravel, wastewater moves to greater depths within a short time. This reduces the contact time for treatment and the wastewater can potentially pollute the groundwater.

For optimum wastewater treatment, the fundamental principle is to promote unsaturated flow of wastewater through capillary pores in order to increase retention time for biochemical reactions and bacterial die-off (Reddy et al., 1981; Loomis 1996). This is controlled by pore size distribution, soil structure, soil texture and organic matter content (Van Elsas et al., 1991; Fontes et al., 1991; Yee et al., 2000). Thus, the draft 2008 draft revisions of Alberta Private Sewage Systems Standard of Practice 1999 Handbook (Safety Codes Council, 2000) makes adjustments by reducing the loading rates on coarse textured soils based on the concept of travel time (Tyler and Mokma, 2004).

1.4 Loading Rates and Wastewater Treatment

The loading rate of the effluent is one of the most important controls on the movement of water in soil. When loading rate is low, the soil gets enough time to renovate the wastewater. Initial estimates of the loading rate were made from percolation tests obtained from a soil infiltration area, however research in the past 40 years has

shown that percolation tests are quite variable and have to be supplemented or completely replaced by other methods which are based upon soil physical properties such as texture, structure and consistency (Brown et al., 1994; Gross et al., 1998). Thus, Tyler and Mokma (2004) calculated loading rates based on soil texture which also result in the retention of the wastewater in soil for treatment. Unsaturated flow of effluent through micro- and meso- pores increases the efficiency of microbial inactivation due to slower average pore water velocities and increased surface contact per net distance traveled. In contrast, under wet or saturated conditions, water flows through macropores, cracks and channels that results in short circuiting of the treatment of wastewater as it rapidly moves through the soil.

1.5 Methods of Application and Treatment of Wastewater

In the at-grade, soil-based dispersal systems, perforated PVC pipes of 25 mm to 38 mm diameter with small 3.2 mm orifices typically spaced at 60 or 90 cm are being used to distribute wastewater along the laterals through pressure distribution. In some earlier designs orifice spacing was as much as 150 cm apart. This is an improvement over gravity distribution methods where localized loading is very heavy, however it still results in point application of the effluent into the soil at each orifice. The spacing of the orifices can significantly impact the amount of point loading. This method of application is under question (Juma et al., 2007) because consequently and better alternatives for application are being sought. Well-structured soils receiving wastewater by point application can transmit large amounts of wastewater through the preferential flow paths. In such cases only a small amount of wastewater is in contact with the soil volume and the beneficial effect of moving wastewater through micro- and meso-pores by capillary flow is lost.

Drip irrigation has been used in agriculture to increase water use efficiency and this technique is now being used for wastewater dispersal into the soil. The drip distribution system consists of several lines of small diameter, flexible, polyethylene tubing spaced 60 cm apart with emitters at 30 or 60 cm within each branch. This allows the wastewater to be dosed in very small amounts over a large volume of soil, thus providing an optimum

environment for the soil and its organisms to treat it before it goes to the deeper depths. This technique also reduces wastewater transport via preferential flow.

Recently, Brilliant Blue food dye has been used to track the water movement and preferential flow paths (Neurath et al., 2005; Bundt et al., 2001). Brilliant Blue FCF has been used in several field experiments due to its good visibility, low toxicity, and weak adsorption on soil. Soils with higher clay and low organic matter content tend to absorb more dye than others. A dye concentration within the range of 3-5 kg/m^3 is recommended for good stain visibility (Flury and Fluhler, 1994). Therefore, this technique has a potential of being used to track wastewater movement through soil pores.

1.6 Methods of Application and Transport of Nutrients and Organisms

Hagedorn et al. (1978) have observed rapid transport of bacteria and viruses through the soil under saturated flow conditions. Under these conditions, the matric potential approaches zero and water is drained from the macropores by the force of gravity. The pore size distribution controls the transport of microorganisms through the soil (Wong and Griffin, 1976; Worrall and Roughley, 1991). In contrast, under unsaturated conditions, the average pore water velocities decrease and the surface contact per net distance traveled by the organisms increases. Thus, virus inactivation in soil columns is higher under unsaturated conditions compared to saturated conditions (Yanjie et al. 2003; Lance and Gerba, 1984; Lance et al., 1976). Brown et al. (1979) reported that most fecal coliform bacteria and coliphage viruses were removed within the first 30 centimeters travel through an unsaturated soil.

1.7 Comparison of Point and Drip Methods of Application

Drip dispersal method has many advantages over the point dispersal method (Fig. 1.2). Benefits of drip include: (1) a greater control of application of wastewater through emitters; (2) an increase the soil water retention (Jnad et al., 2001) which also results in reduction of pathogens and nutrients (Enriquez et al., 2003; Bohrer and Converse, 2001); and (3) conservation of water (Schleiche, 1977).

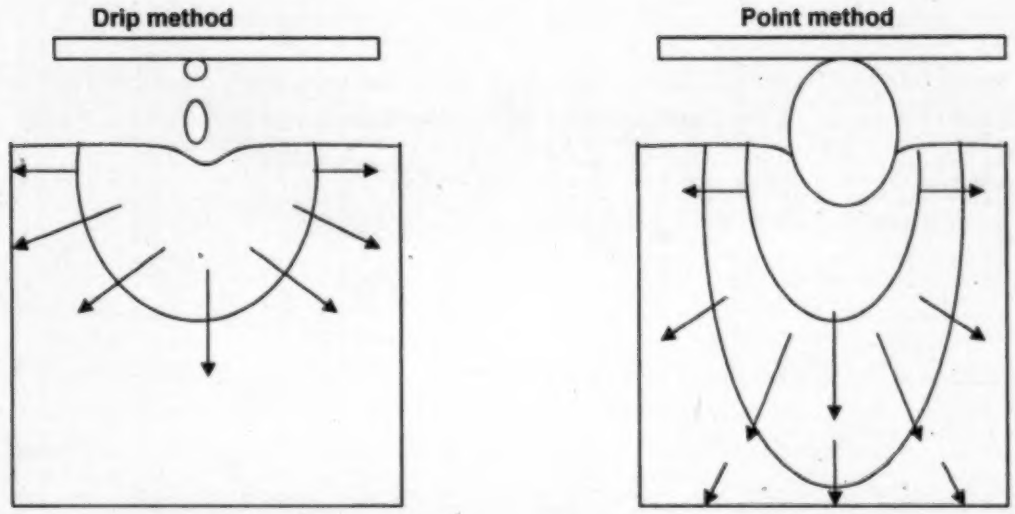

Figure 1.2. Point and drip irrigation in soil (adapted from Crops, Soils, Agronomy CSA News, Sept 2008 V53 No.9).

1.8 Research Objectives

The objectives of this research were:

a. to compare the movement of water in soil columns receiving wastewater by point and drip application methods (Chapter 2).

b. to assess lateral and vertical movement of water, nutrients, fecal coliforms and somatic coliphages in soil columns receiving wastewater through point and drip application methods (Chapter 3).

c. to determine the water distribution pattern, behavior of nutrients, fecal coliforms and somatic coliphages in soil receiving wastewater through point and drip application methods under field conditions (Chapter 4).

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Chapter 2

Lateral and Vertical Movement of Water in Soil Columns Receiving Wastewater by Point and Drip Application Methods

2.1 Introduction

In contrast to the conventional septic tank and subsurface, gravity-fed septic field systems which have been used around the world for more than a century (Beal et al., 2005), at-grade pressurized systems have an aerobic treatment unit (ATU) or advanced treatment plants (ATP) and at-grade dispersal fields. These systems have been developed for the dispersal of secondary treated effluents on to the surface of forest soils for final treatment of the wastewater. In theory, the surface and subsurface soil horizons should renovate the ATU- or ATP-treated secondary effluent before it enters the groundwater.

In at-grade dispersal fields, the distribution piping is pressurized and placed on the surface of the ground within a "natural" forested area. To eliminate the freezing effect in winter season the distribution piping is covered by an open bottom chamber or a 30 cm (12") or larger "half-pipe" that provides a shielding housing that is further covered with wood chips and/or shredded tree cuttings appropriate for the ecology of the site. The intent of the system design is to deliver the effluent uniformly along the pressurized lateral pipe through orifices which are generally located at regular intervals.

In at-grade dispersal systems, effluent is applied to the soil through orifices which are commonly located at 60 to 90 cm intervals along the laterals. Therefore, as the effluent gets pumped through the orifice, such dispersal systems are referred to as point application systems. Juma et al. (2007) found that in point application systems fecal coliforms could be detected at up to a soil depth of 60 cm. In contrast to these systems which rely on point application, drip irrigation systems, which were initially developed for irrigation of agricultural crops, are now being used to disperse wastewater. These systems are precise, high frequency, low-volume dosing systems which reduce the hazards of waterlogging and excessive percolation (Hillel, 2008). Bohrer and Converse (2001) shown that drip application of sewage effluent resulted in its retention in the upper soil horizons. This also promoted its treatment in soil.

As the transport of microorganisms and nutrients with wastewater is largely related to the saturated and unsaturated flow of water as well as soil water content, it is important to understand the hydraulic behavior of wastewater in soil in order to reduce the transport of pathogenic microorganisms and nutrients into the groundwater (Reneau et al., 1989 and 1985; Stotzky, 1985; Brown et al., 1977; Schijven, et al., 2002; Powelson and Mills, 2001; Lo et al., 2002).

2.2 Literature Review

The easiest way to examine the differences between point versus drip application of wastewater is by studying the following figure that represents many years of research of agricultural irrigation systems (Fig. 2.1).

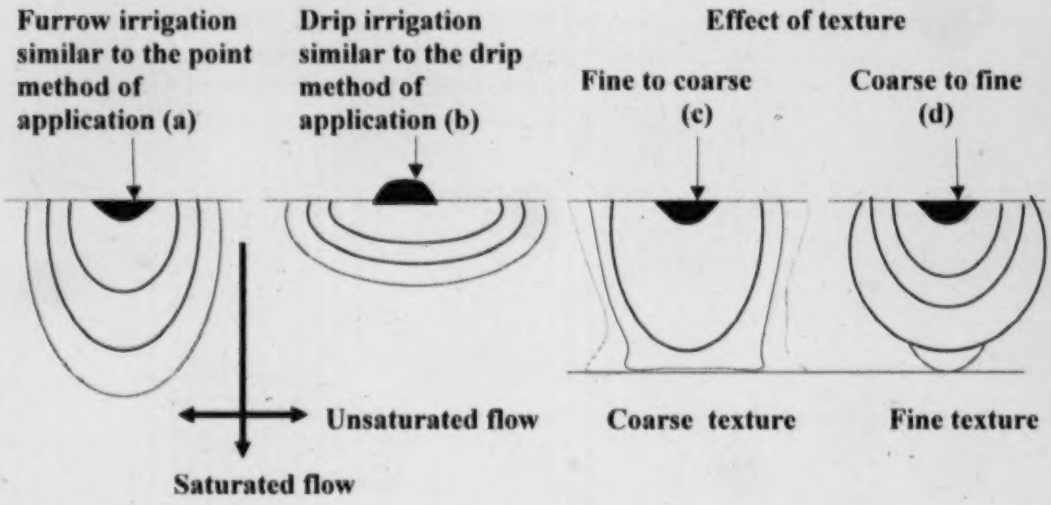

Furrow irrigation similar to the point method of application (a)
Drip irrigation similar to the drip method of application (b)
Effect of texture
Fine to coarse (c)
Coarse to fine (d)
Unsaturated flow
Saturated flow
Coarse texture
Fine texture

Figure 2.1. Movement of water in furrow and drip irrigation (adapted from Hassett and Banwart, 1992)

Rapid water application from furrow irrigation (Figure 2.1a) is almost equivalent to point application of sewage effluent and causes saturated flow resulting in rapid transport of wastewater through surface soil horizons and deeper percolation of wastewater. In contrast, slow application of wastewater through drip irrigation favors capillary flow which results in the lateral flow of wastewater and more uniform wetting of the surface soil (Figure 2.1b). Retention time of wastewater is greater in the drip versus the point

application method. Textural discontinuity is very common in soils. In the case of a fine-textured soil horizon overlying a coarse textured horizon, the wastewater has to be above field capacity at the soil horizon interface before it can enter the coarser textured horizon (Figure 2.1c). In contrast, in the case of a coarser textured horizon overlying a finer textured horizon, infiltration is rapid through the surface horizon and much slower in the underlying soil horizon. This generally results in lateral flow.

In a saturated soil, the positive pressure potential is the driving force of water through macropores but, in unsaturated flow, water moves through meso- and micro-pores and along surfaces of the soil particles by capillary forces or by differences in matric potential. Movement of water by unsaturated flow is much slower than by saturated flow. In transient flow, the moisture content (θ) and matric potential (ψ) are variable over space and time. The flow can be either saturated or unsaturated. Under steady state conditions, θ and ψ are constant over space and time. The flow can be saturated or unsaturated.

Examples of the above are also represented in Fig. 2.1a. In furrow irrigation or in point application system, water is ponded so wetted soil is at saturation (θs), but there is a variable θ and ψ at the wetting front as it moves through the soil. In drip application, Fig 2.1b, there is no ponding so the soil is unsaturated, but there are still variable θ and ψ at the wetting front as it moves through the soil. The mathematics of these types of water flow patterns in soil have been extensively described by Darcy's Law for saturated flow, the Darcy Buckingham equation for unsaturated flow and Richard's continuity equation for transient flow. Excellent coverage of these concepts can be found in a number of Soil Physics texts e.g., Hillel (1998).

In order for water to actually move from one point to another, two conditions must be met. First, there must be a difference in hydraulic head between the two points (that is, ΔH must be greater or less than zero). Second, the soil between these two points must be permeable enough to allow the movement of water. Hydraulic conductivity (K) is a measure of this ability of a soil to transmit water. The larger the K value of a soil, the greater will be the movement of water through it for any given hydraulic gradient. The main driving force of water movement in saturated and unsaturated flow are the potential gradient and hydraulic conductivity.

Stotzky (1985) found that the unevenness in soil water content, soil texture, and soil structure affected bacterial transport and retention mechanisms in both horizontal and vertical directions. Powelson and Mills (2001) observed that total bacterial cell concentrations in the saturated outflow was significantly lower in case of constant unsaturated flow than under constant or variable saturated flow. They also reported that the air-water interfaces in unsaturated porous media are important bacterial adsorption sites. Viruses also have been observed to interact with the air-water interfaces (Powelson et al., 1990; Poletika et al., 1995).

Movement of organisms and nutrients largely related to the movement of water, therefore, it is important to understand the water movement in soil in both vertical and horizontal directions. This could lead to better understanding of the effect of dose volume, loading rate and treatment of wastewater in soil.

2.3 Objective

The objective of this research was to quantify the lateral and vertical movement of water in soil columns receiving wastewater by point and drip application methods.

2.4 Material and Methods

This section describes the procedures for the collection of soil samples from Rocky Mountain House (Alberta), collection of primary effluent (PE) from the Goldbar wastewater treatment plant in Edmonton (Alberta), construction of soil columns from polyvinyl chloride (PVC) pipe, packing and instrumentation of specific sections of columns and application of water followed by diluted (PE) over periods of 60 and 90 days.

2.4.1 Experimental soil:

As the at-grade, onsite, private sewage treatment systems work primarily in forested environments, the experimental soil was a Dark Gray Luvisol collected from Rocky Mountain House in Alberta, Canada. The major soil horizons of this soil were Ah (0-22 cm), Ae (22-25 cm), Bt (25-52 cm), BC (52-85 cm) and Ck (85-100+ cm). The bulk densities of Ah, Bt, BC and Ck were 0.92, 1.27, 1.24 and 1.30 Mg/m^3, respectively.

Soil samples from three transects were taken at 10 cm intervals to a depth of 40 cm and at 20 cm depths from 40 to 100 cm from two sides of an opened pit which was almost 3 m in length. The physico-chemical properties of the soil for the 7 soil layers are shown in Table 3.1.

The soil was sieved and passed through 2 mm sieved and packed with 5 cm increments to obtain desired soil bulk density and to eliminate preferential flow through macropores

The soil pH was in the acid range and increased with depth. The soil was not saline. Clay content increased from 23 to 55% with depth, therefore the textural classes were silt loam, silty clay loam and clay, respectively. The total organic C content of the top 20 cm was between 3.86 and 3.70% and decreased sharply in the 20 to 40 cm interval and then increased to about 1.75% in the 40 to 100 cm depth. The total soil N content showed a trend that was similar to total organic C (Table 2.1).

Table 2.1. Physical and chemical properties of Dark Gray Luvisol from Rocky Mountain House, Alberta.

Depth cm	pH	EC	Sand	Silt	Clay	Texture	Total C	Total N
		(dS/m)		%			%	%
0-10	4.84	0.053	23	54	23	SiL	3.86	0.32
10-20	4.94	0.053	22	53	25	SiL	3.70	0.31
20-30	4.95	0.053	22	48	30	SiCL	0.88	0.10
30-40	5.05	0.034	22	35	43	C	0.79	0.09
40-60	6.84	0.186	15	38	47	C	1.74	0.12
60-80	6.92	0.188	9	36	55	C	1.75	0.12
80-100	6.91	0.188	8	37	55	C	1.75	0.12

2.4.2 Effluent collection and preparation:

Grab samples of untreated effluent which is also known as primary effluent (PE) were taken every Tuesday and Friday for 12 weeks by staff at the Goldbar wastewater treatment plant in Edmonton and stored at 4°C. The 8L samples were stored in sterile 20L polypropylene containers and brought to the Onsite Wastewater Treatment Research Laboratory, University of Alberta. All analyses of the PE were performed within 24 hours of sample collection.

Test results of PE from the laboratory at Goldbar Wastewater treatment Plant showed that the strength (in terms of TSS, BOD5, total and fecal coliforms and nutrients) of the PE from Goldbar was much higher than the strength of secondary treated sewage effluent from effluent holding tanks of several ATPs (Juma et al., 2007). Therefore, a decision was made to dilute the PE 5-fold with distilled water. A secondary benefit of this decision was the reduction of chances of getting a formation of biomat in soil columns because the absence of biomat was observed in at-grade systems receiving secondary treated effluent in the field (Juma et al., 2007). After dilution, the strength of diluted primary effluent (DPE) was equivalent to that of secondary treated effluent from ATP. The PE and DPE were analyzed for pH, BOD_5, EC, NH_4^+, NO_3, and extractable PO_4^{3-}, fecal coliforms and somatic coliphages by standard methods which will be described in a greater detail in Chapter 3. The chemical and microbiological properties of the primary and diluted effluent are given in Table 2.2. The data for PE obtained in our lab were similar to the daily and monthly averages provided by the analytical laboratory at the Goldbar wastewater treatment plant.

The 5-fold dilution did not give the 5:1 ratio for all the chemical and microbiological properties of the effluent because it is not a homogenous mixture like the laboratory graded chemicals. The main objective was to reduce the strength of PE and make it similar to a secondary treated effluent from an ATP.

Table 2.2. Chemical and microbiological properties of primary and diluted effluent.

Type	pH	EC dS/m	TSS mg/L	BOD_5 mg/L	Fecal Coliforms Log MPN/100 ml	Somatic Coliphages pfu/100 ml	NH_4-N mg/L	NO_3-N mg/L	PO_4 mg/L
Primary	7.09	1.20	80	130	7.36	1500	108.65	0.60	20.01
Diluted (1:5)	6.64	0.39	21	30	5.04	250	20.12	0.10	3.54

2.4.3 Column setup:

Eight columns were made of polyvinyl chloride (PVC) pipe (100 cm in length, 20 cm diameter). The columns were cut into five sections (20 cm in length) for the convenience

of sampling at the end of the experiment. The sections were glued together with silicon gel and duct tape was applied around the PVC column at the sealed joints. Then the column was set up on a PVC cap which was set in wooden frame. A hole (6 mm diameter) was drilled at the bottom of the PVC cap and a nozzle (4 mm diameter) was installed to collect the leachate from the column. The cap was lined with glass wool with a thin layer (5 mm) of sand added on top of the glass wool (Figure 2.2; Photo 2.1). The columns were then packed with three horizons of soil in 5 cm increments and packed to a bulk density to simulate field conditions. The three layers corresponded to Ah (0-20cm), Bt (20-50 cm) and BC/Ck (50-100 cm) horizons. The soil column was packed from bottom up, thus the 50-100 cm layer had a bulk density of 1.30 Mg/m^3 (g/cm^3); the 20-50 cm layer had a bulk density of 1.27 Mg/m^3; and the 0-20 cm layer had a bulk density of 0.92 Mg/m^3. As the soil in columns was repacked, the amount and continuity of macropores which occurs under field conditions, could not be reproduced.

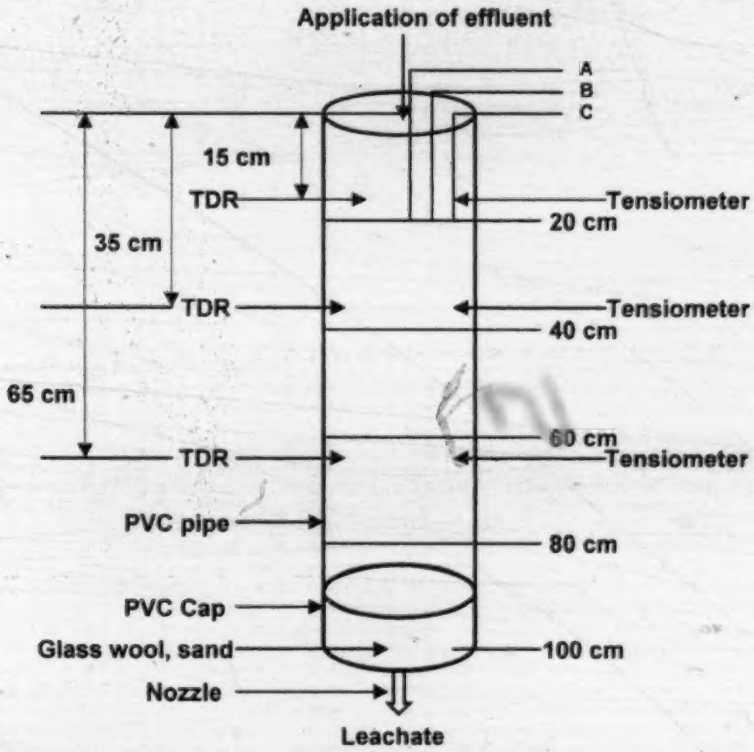


Figure 2.2. Schematic diagram of soil column

2.4.4 Instrumentation:

In order to track the direction of water flow vertically, three elbow tensiometers were installed in each column at depths of 15, 35, and 65 cm from the top. In order to track the volumetric moisture content, three Time Domain Reflectometry (TDR) probes were also installed horizontally at the same depths (Figure 2.2). Tensiometers were used to determine the direction of water flow and TDR probes were used to measure the soil volumetric moisture content nondestructively.

Three TDR probes were also inserted vertically from the top of the column to a depth of 20 cm. These were used to measure moisture content laterally and were inserted in the central core (6.6 cm diameter), first torus (6.6 cm diameter) and second torus (6.6 cm diameter). These are represented by letters A, B, and C respectively, in Figure 2.2 and also shown in Figure 3.6.

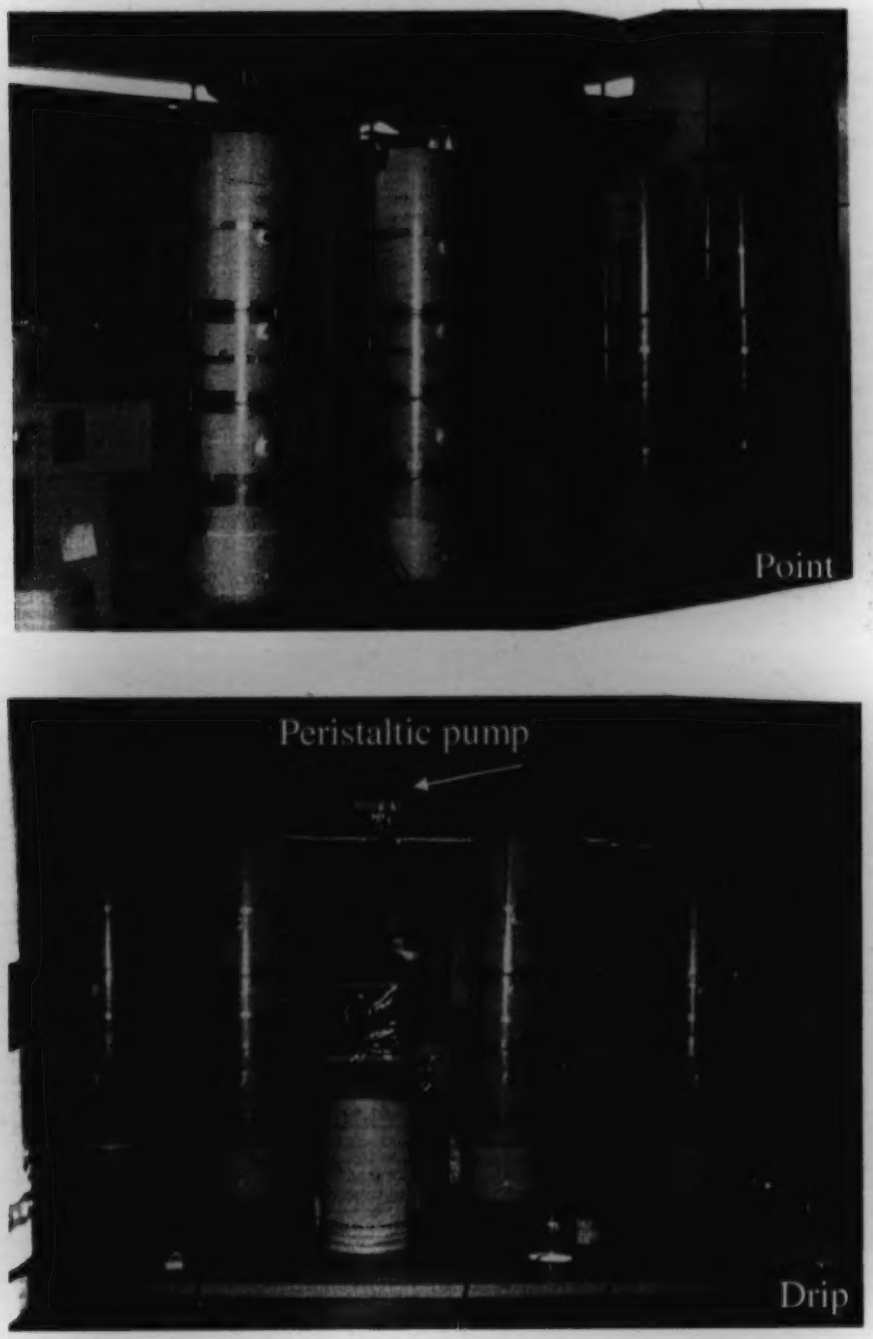


Photo 2.1. Photographs of point (above) and drip application systems (below) in the laboratory with peristaltic pump.

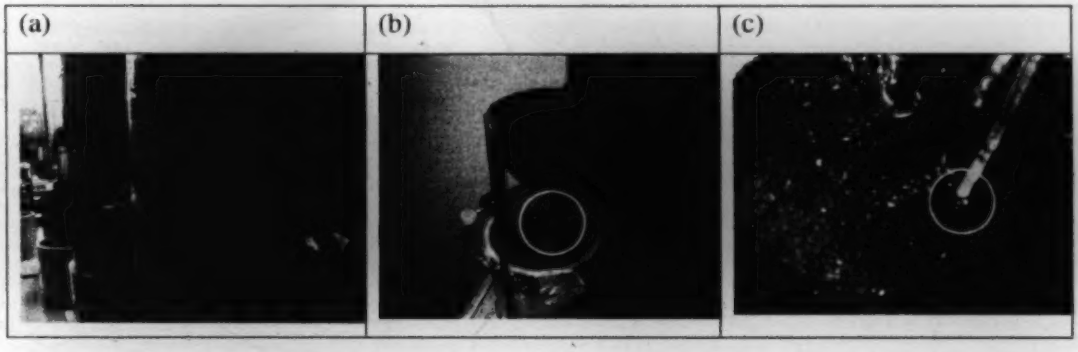


Photo 2.2. A close up of an installed elbow tensiometer and a TDR probe into the soil column are shown in (a), point application and drip application methods of applying DPE are shown in (b) and (c), respectively.

2.4.5 Methods of DPE application:

Two methods were used to apply DPE to the soil columns. The details are as follows:

Point application:

Diluted primary effluent was applied through a funnel (diameter 5 mm) in the centre of the column and the leachate collecting tube was transparent with 4 mm diameter.

Dose volume: 500 ml; Flow Rate: 500 ml/min; Dose: 1/day;

Dose duration: 1 minute; Duration of the experiment: 60 days and 90 days

Drip application:

A peristaltic pump was used to deliver the prescribed DPE dose (located at the top; Photo 2.1) from an orifice (2 mm diameter) located in the middle of the infiltrative surface at 1.66 ml/min to yield a dose volume.

Dose volume: 500 ml; Dose flow rate: 1.66 ml/minute;

Dose duration: 300 minutes (5 h); Duration of the experiment: 60 days and 90 days

The loading rates for both methods were 15.9 L/m^2/day (0.336 US gal/sq. ft/day; 0.279 Imp. gal/sq. ft/day) and correspond to those recommended by Tyler and Mokma (2004).

2.4.6 Application of Diluted Primary Effluent (DPE):

Before adding the effluent to the columns, distilled water was added for one week to see the performance of water movement through the column and collected leachates, which were not analyzed. Then DPE was added for the first week to displace the distilled water from the columns and leachates were not analyzed from the viewpoint that distilled water diluted the concentration of nutrients. The leachates were collected from the second week onwards and used for chemical and microbiological analysis. The results of these measurements will be presented in Chapter 3 because the focus of this chapter is lateral and vertical movement of wastewater in soil columns.

2.4.7 Leachate collection:

Leachate was collected in sterilized glass containers from each column on every day and were analyzed on Wednesdays and Saturdays over the duration of the experiment. The leachates were stored at 4^0C and were analyzed for NH_4^+, NO_3, and extractable PO_4^{3-}, fecal coliforms and somatic coliphages by standard methods which will described in detail in Chapter 3. The results of these measurements will be presented in Chapter 3.

2.4.8 Tensiometer Readings and TDR Probe Readings:

Three elbow tensiometers were installed in each column at 3 different depths (15, 35, and 65 cm). Tensiometer data was measured with a Tensimeter (Soil Measurement Systems, Tucson, AZ, USA). The horizontal part of this tensiometer was 9 cm long (including the porous cup). The vertical part, with the septum stopper closure, was 5.5 cm long. Tensiometer readings were read by Tensimeter with 1 millibar sensitivity and were taken once in a day. The TDR probe reading was taken by Metallic TDR Cable Tester (Tektronix 1502B) once in a day. Although all the data were recorded on a daily basis, averages over 5 days are presented in the graphs for the duration of the experiments (60 and 90 days) so that the graphs become more clear with standard deviations. The data for two columns were averaged and the mean and standard deviations are presented in graphs. The columns which were sacrificed at day 60 and day 90 for point and drip application methods will be referred to as P-60 and D-60, and P-90 and D-90, respectively.

2.4.9 Moisture Retention Curves

Soil moisture retention curve of three different layers of soil columns were measured with Pressure Plate Apparatus (Soil moisture Equipment Corp., Model No.1500 F1) at 0.1 Mpa, 0.5 Mpa and 1.5 Mpa pressures. The model used to fit to the data was the one published by Campbell (1974):

$$\theta = \theta_s \left(\frac{\psi_e}{\psi_m} \right)^{\lambda}$$

where θ is the volumetric water content, θ_s is the saturated water content, ψ_m is matric potential and ψ_e is the air entry potential (the potential at which the largest pores in the soil drain) and λ is the Campbell lambda. As the soil textures of the three soils ranged between silty loam and clay, the θ_s was assumed to be 0.42 when $\psi_m = 0$ for all three layers. Under this constraint, the model predicted the moisture retention curves for these soil layers by optimizing the values of ψ_e and λ.

2.4.10 Experimental Set Up, Statistical Design and Sampling Concept

Eight laboratory soil columns were set up and packed with sieved (2 mm) soil from major soil horizons of Dark Gray Luvisol soil sampled from Rocky Mountain House, Alberta as described in Chapter 2 (Table 2.1).

The experimental design consisted 8 soil columns [2 replicates x 2 methods of sewage effluent application (point vs. drip) x 2 durations (60 vs. 90 days)]. However, when the columns were sacrificed, the soil was divided into 6 depths (0-10, 10-20, 20-35, 35-50, 50-75 and 75-100 cm) and sampled laterally into three layers (central core, first torus and second torus). Therefore, in order to statistically analyze all the data together, a complex split plot design was used which consisted of 2 replicates x 2 application methods x 2 durations x 6 depths x 3 lateral layers for each depth.

Thus, the statistical model had 3 sizes of experimental units. The soil column was the experimental unit to which treatments (point versus drip methods of effluent application) and duration of experiment (60 vs. 90 days) were applied. Treatments and durations were arranged within soil columns in a completely randomized design structure. The section of

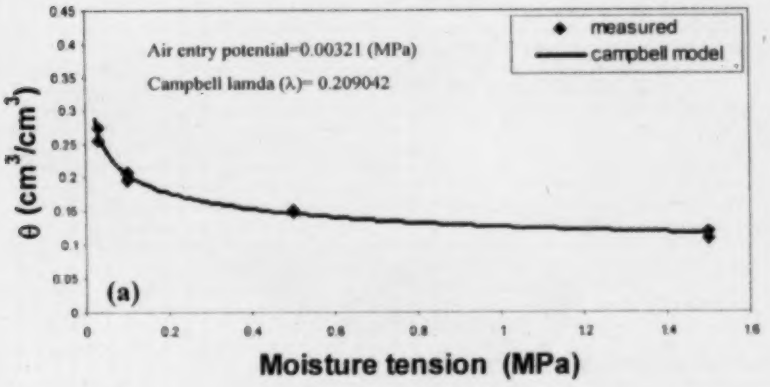
a column (different depths) was the experimental unit for depth, and within a section of a depth was the experimental unit for lateral layers. Dr. George A. Milliken, Department of Statistics, Kansas State University assisted in developing the SAS statistical model.

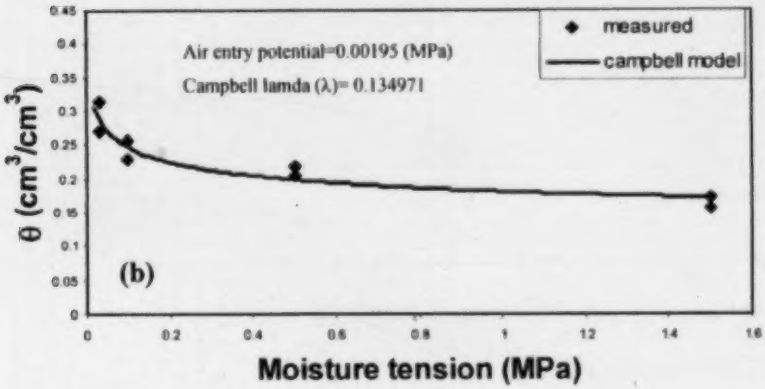
The procedures for collecting primary effluent (PE) from Goldbar wastewater treatment plant and preparation of diluted primary effluent (DPE) have been described in Chapter 2. Results of the analysis of PE and DPE are presented in Table 2.2. The procedure for setting up and packing the columns has also been described in Chapter 2.

2.5 Results

2.5.1 Moisture Retention Curves:

As the soil texture for the Ah, Bt, and BC/Ck layers varied between silt loam and clay, the moisture content at field capacity increased from 27% to 40% (Figure 2.3a-c). The moisture content at the wilting point for the Ah horizon was lower than that in the Bt and BC/Ck layers (Figure 2.3a-c). These trends are consistent with the impact of soil texture/structure on moisture retention curves (Jnad et al., 2001). Although these soil textures are in a close range, there was textural discontinuity in the soil columns and there were differences in bulk density between the soil three layers. Therefore, there is a resemblance between the set up in the soil columns and field conditions. However, as the soil samples in the columns were sieved and repacked, it was quite obvious that the continuity of larger pores was reduced. Under field conditions, plant roots were present in the surface horizons and earthworms were present in the Bt and BC/Ck horizons. However, the bulk soil was fine textured (Table 2.1.)





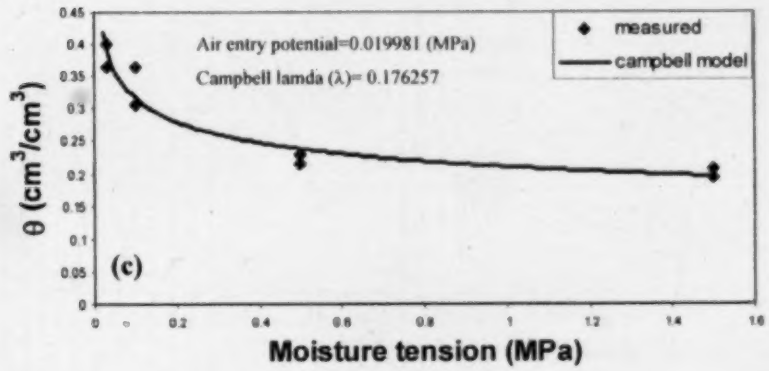


Figure 2.3. Moisture retention curve of Ah (a), Bt (b), and BC/Ck (c) layers of the Dark Gray Luvisol.

2.5.2 Soil Water Content (Θ) measured with TDR probe and Soil Water Suction (Ψm) measured with tensiometer:

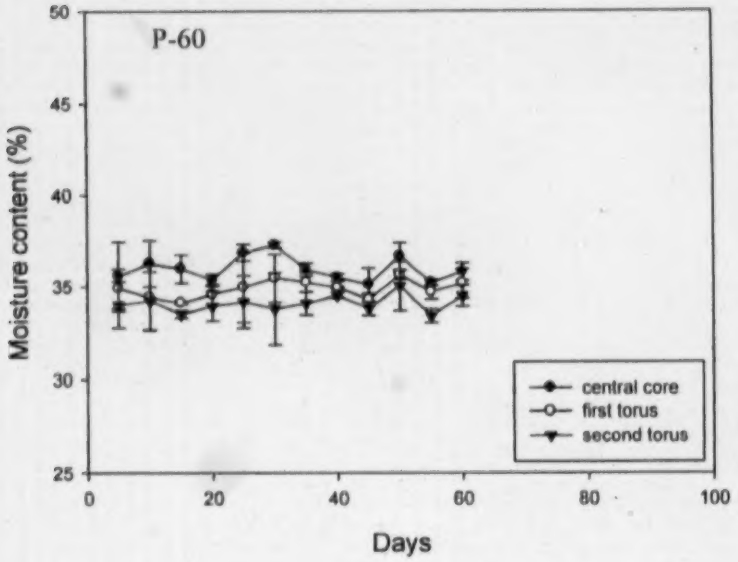
Volumetric water content (%) was directly measured with the TDR probes during the 60- and 90-day experimental periods. The moisture content in three different lateral layers (central core, first torus, and second torus) of 20 cm depth, and three vertical layers at 15, 35 and 65 cm depths in the point and drip application systems of the columns was measured. The five day averages for these measurements are presented in Figure 2.4 and 2.5.

2.5.2.1 Lateral Distribution of Moisture in the 0-20 cm layer

As the overall measurements of volumetric water content in three lateral layers did not vary widely (Figure 2.4 and 2.5), the data for the duration of the 60- and 90-day experiments were averaged and are presented in Figure 2.6.

The average water content up to 20 cm depth for the lateral layers was 38.1% in the central core, 35.3% in the first torus and 34.0% in the second torus for the P-60 (Figure 2.6a). In P-90, the corresponding values were 36.8%, 36.3% 35.3%, respectively (Figure 2.6b). In D-60, the average moisture content was 34.6%, 33.8% and 31.7%, and 34.2%, 33.6, and 31.8% for the central core, first and outer tori, respectively (Figure 2.6) for D-90.

The water content in lateral layers in the 20 cm depth was above field capacity but lower than the maximum water holding capacity during the length of the experiment. The distribution of water was varied from saturated to unsaturated for point and drip application methods. There was a tendency of overall moisture content to decrease with radial distance. As the diameter of the soil column was only 20 cm and the surface layer was dosed once a day, the difference of moisture between the central core and the second torus were small and not significantly different ($P>0.05$) (Table 3.5). Therefore, it was possible to simplify this experiment to a one-dimension flow from the perspective of wastewater flowing through the soil columns. However, these data show that wastewater applied by both the point and drip methods was drawn laterally from the central core to the edge of the columns.



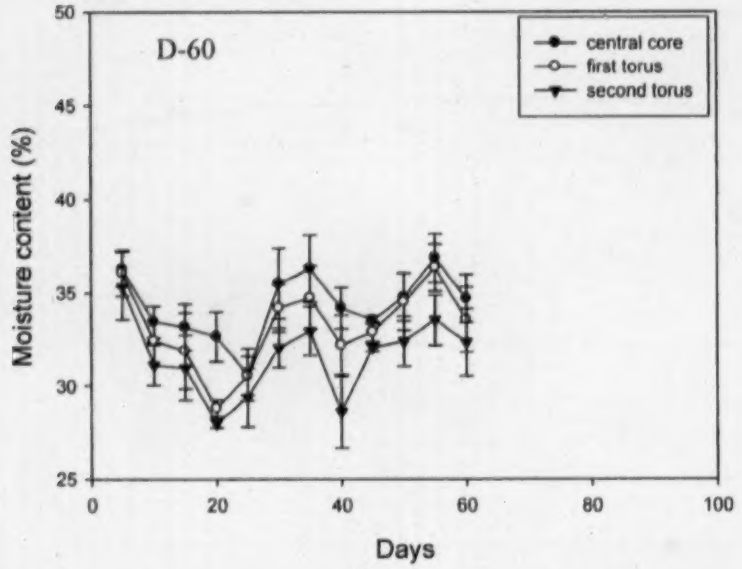
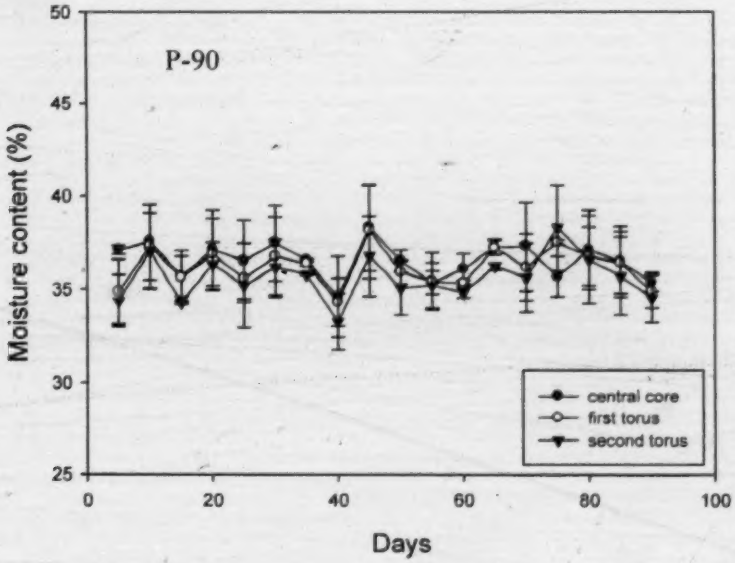


Figure 2.4. Volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 60 days at three lateral layers in the 0-20 cm depth. Error bar shows standard deviation (±).



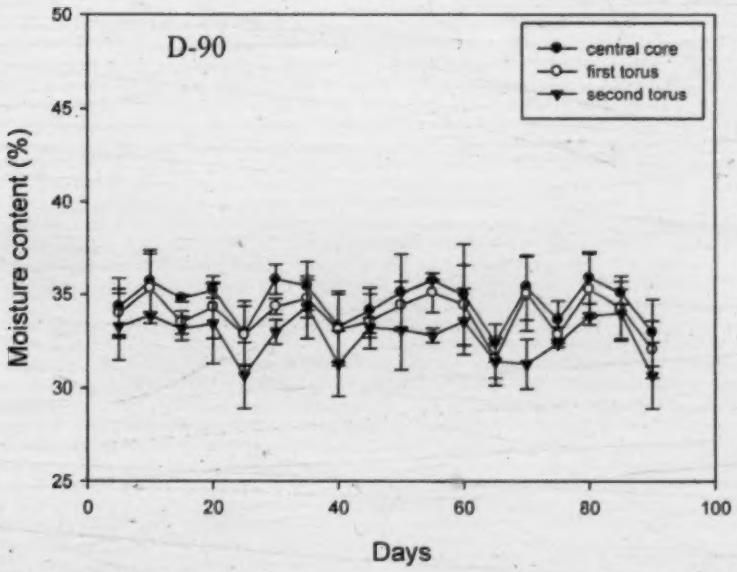


Figure 2.5. Volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 90 days at three lateral layers in the 0-20 cm depth. Error bar shows standard deviation (±).

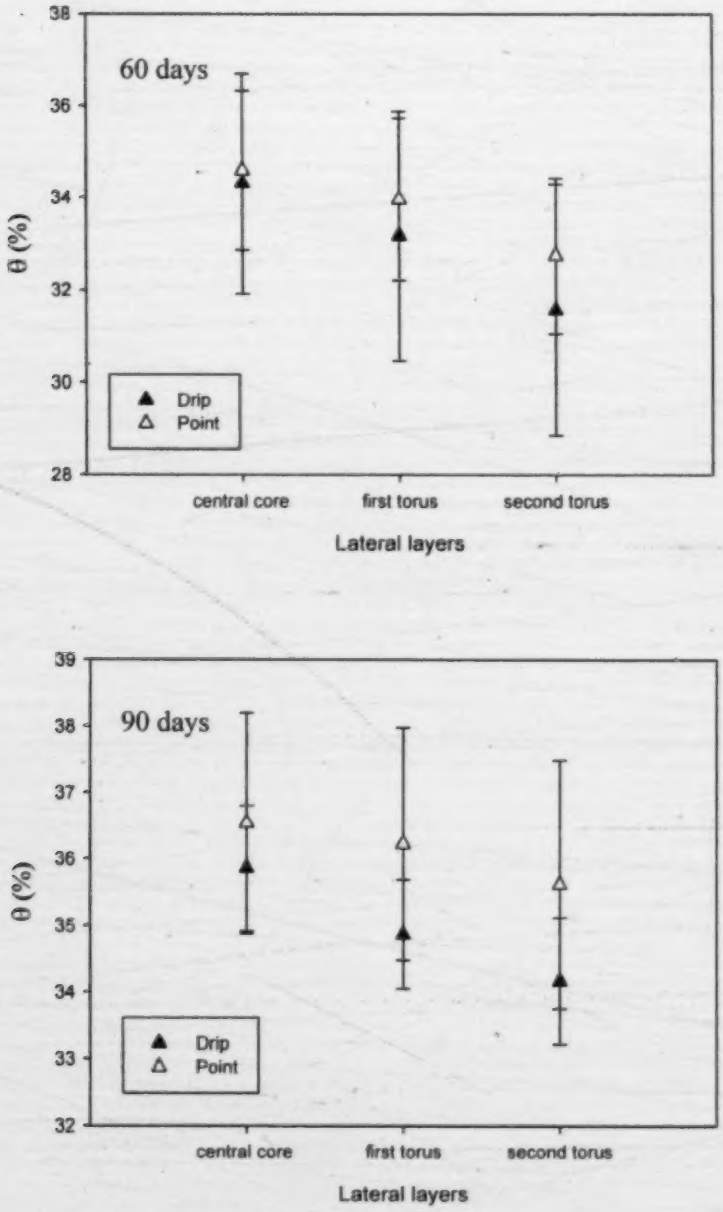


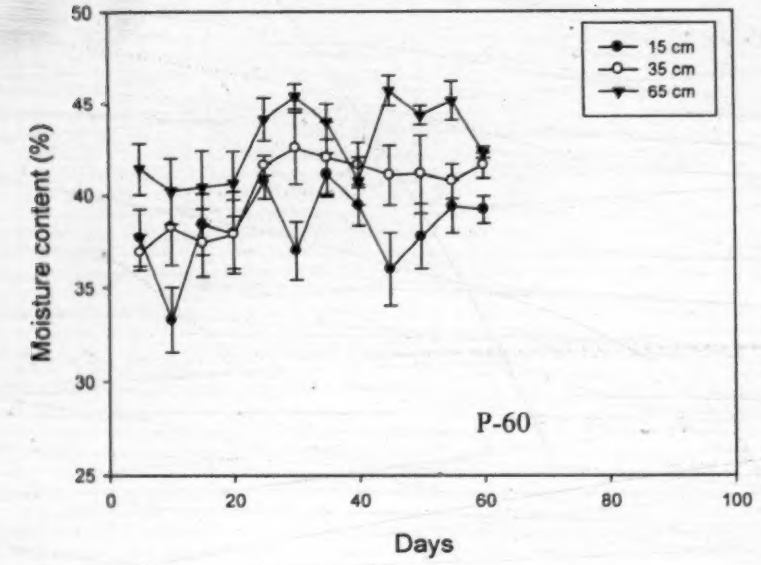
Figure 2.6. Average volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 60 and 90 days at three lateral layers in the 0-20 cm depth. Error bar shows standard deviation (±).

2.5.2.2 Vertical Distribution of Moisture in the Three Layers

The five day averages for volumetric moisture in three vertical layers at 15, 35 and 65 cm depths of P-60 and D-60 obtained with TDR probes are presented in Figure 2.7 and those for P-90 and D-90 are presented in Figure 2.8. The data for moisture potentials obtained with elbow tensiometers are presented in Figures 2.9 and 2.10.

The water content in the columns receiving effluent by the drip method was lower than the columns receiving effluent by the point method (Figures 2.7 and 2.8.). In P-60, the average water content increased with depth and was 39.6%, 41.1% and 42.7% at 15, 35 and 65 cm depths. The corresponding water potential was -0.000126, 0.000374, and -0.000572 MPa, respectively. In the P-90, the average water content at the same depths was 38.4%, 39.8% and 43.0% and water potential was -0.001180, 0.002876, and -0.001286 MPa, respectively (Figures 2.9 and 2.10). In D-60, the average water content in the same depths was 38.6%, 40.4% and 41.8% and water potential was -0.001484, 0.003096, and -0.001612 Mpa, respectively. In the D-90, the average water content at the same depths was 39.4%, 41.9% and 43.5% and water potential was -0.001278, -0.001204, and -0.001614 Mpa, respectively (Figures 2.9 and 2.10).

The water content in three different vertical layers increased with depth. In most cases, the water content in soil layers was below the saturation point when the effluent was applied by the drip method (Figure 2.11). When the effluent was applied by the point method, it was at or above the saturation point (Figure 2.11) for point application system. Water content throughout the experimental period followed a similar pattern in both application systems. The figures of water contents with days for 60 and 90 days (Figures 2.7-2.8) did not fell in the same range because the columns for 60 days are different from the columns of 90 days treatments, therefore, even though the conditions were same in both columns there were some deviations (within 5% range) in the transient flow of water in these columns



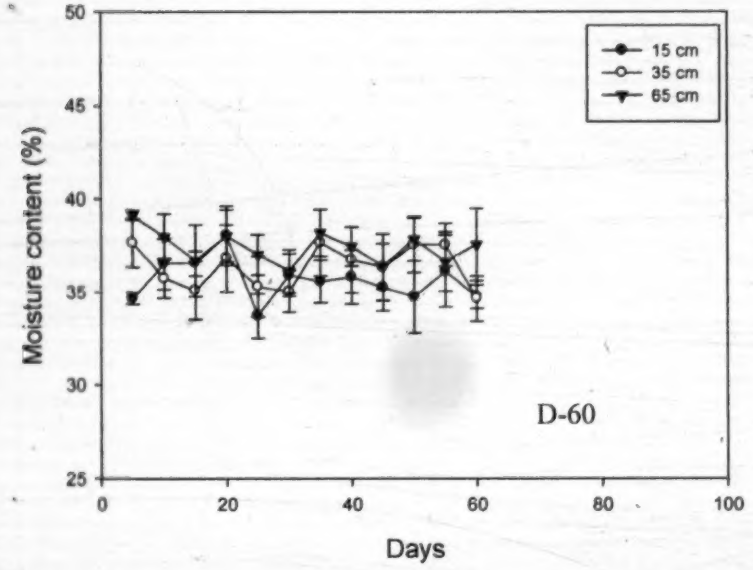
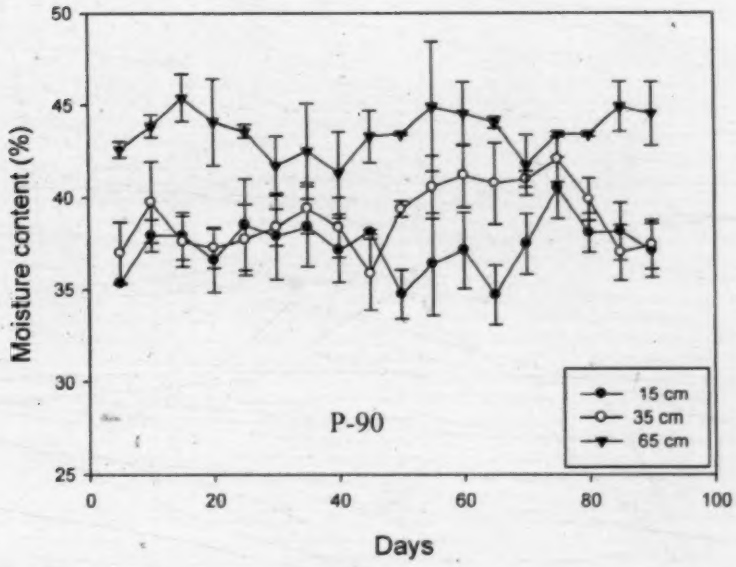


Figure 2.7. Volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 60 days at three vertical depths. Error bar shows standard deviation (±).



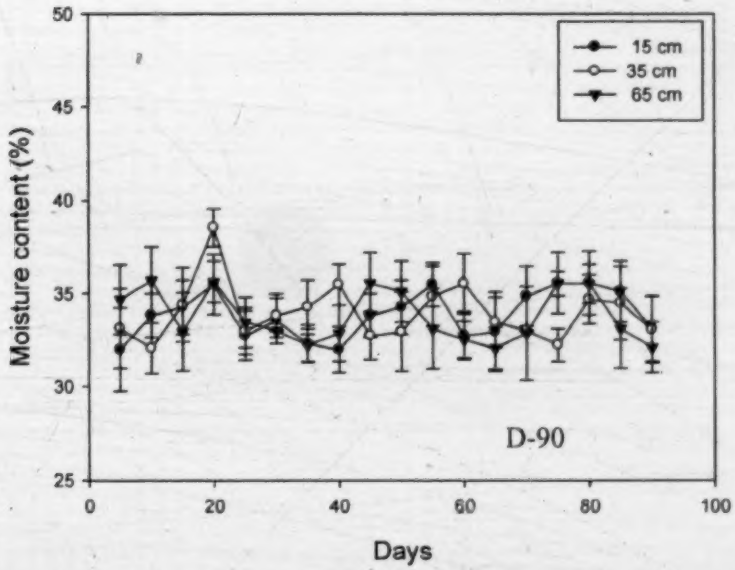
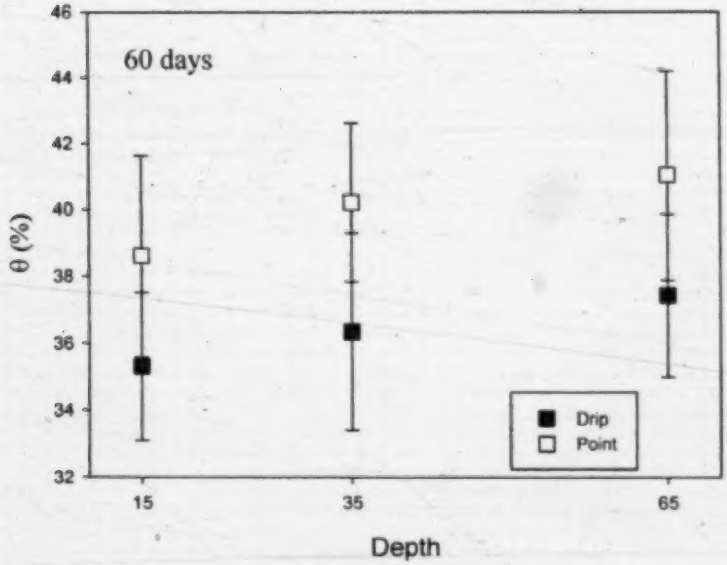


Figure 2.8. Volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 90 days at three vertical depths. Error bar shows standard deviation (±).



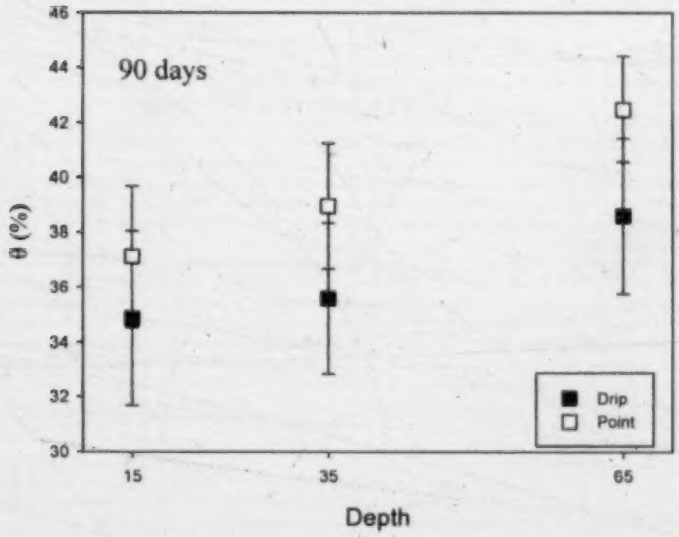
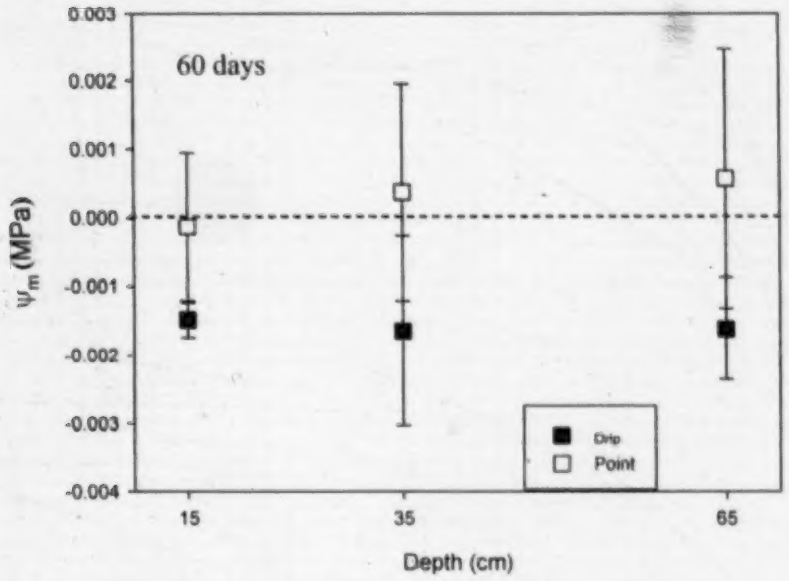


Figure 2.9. Average volumetric water content (θ) in soil columns receiving effluent by point and drip application methods for 60 and 90 days. Error bar shows standard deviation (±).



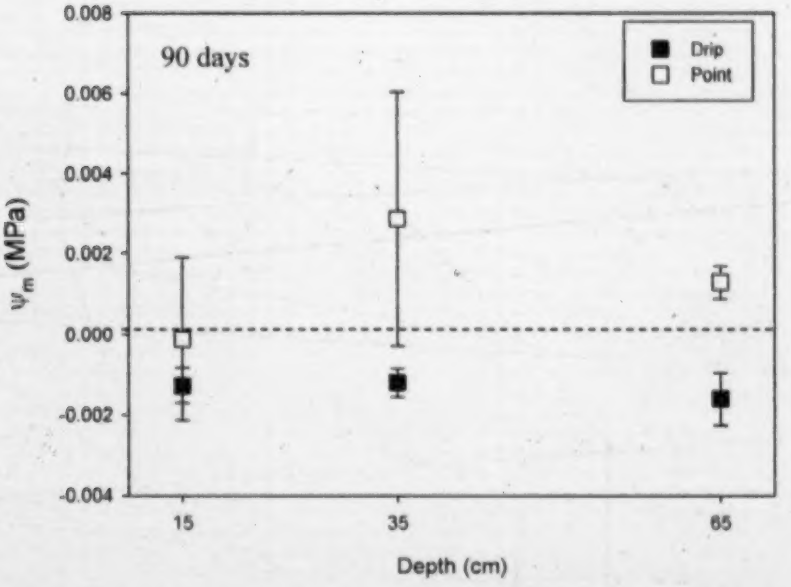


Figure 2.10. Matric potential in soil column in point and drip application of effluent for 60 and 90 days, respectively at three vertical depths. Error bar shows standard deviation (±).

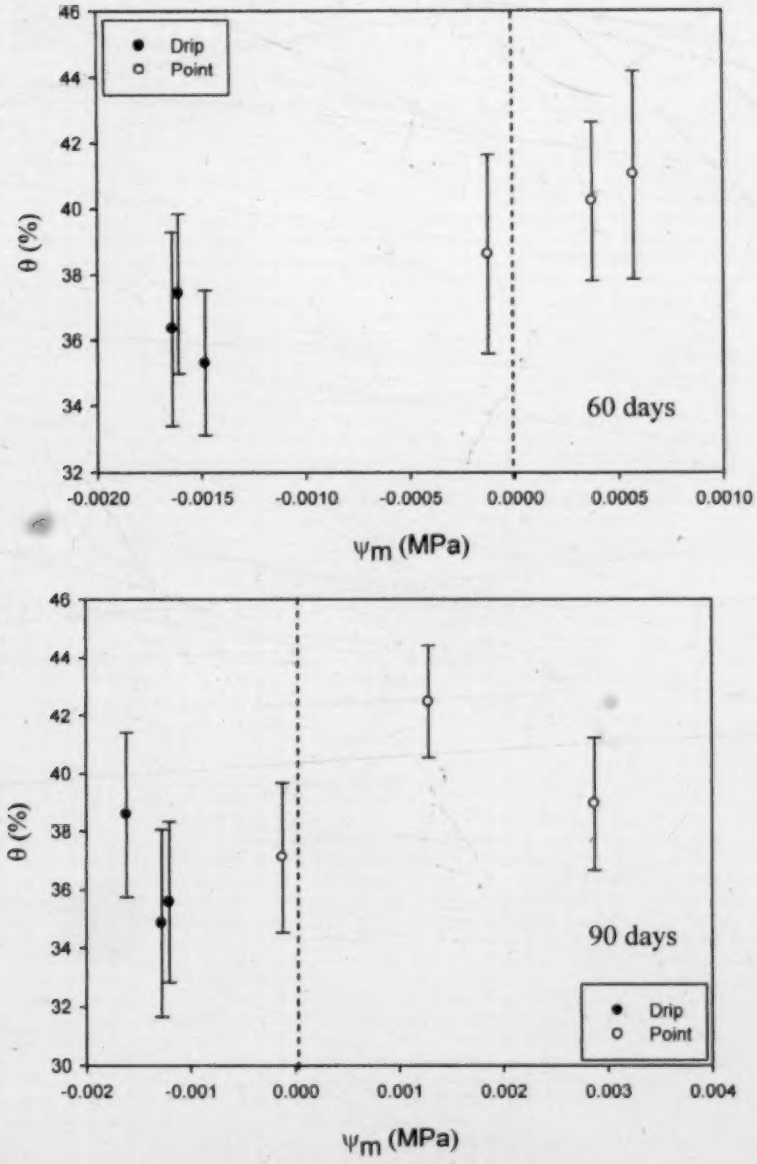


Figure 2.11. Matric potential and volumetric water content in soil columns receiving effluent by point and drip application methods for 60 and 90 days, respectively in three vertical depths. Error bar shows standard deviation (±).

2.6 Discussion

This experiment was conducted to address the fundamental question of point application of sewage effluent to soil which will allow soil more time to treat wastewater and reduce the groundwater contamination. The experiment was design to mimic the dynamics of effluent being applied from an orifice in a lateral of an at-grade, soil-based dispersal system and that of effluent being applied through a dripper from a drip line. The issue of textural discontinuity between soil horizons, which is a natural phenomenon, was also addressed by layering of soil materials from different horizons as they occurred in the field. The results of this experiment can be interpreted as follows:

2.6.1 Lateral Flow

The water content at the same depth in three different lateral layers was similar in this study because the distance between the three lateral layers were not wide enough to show any variations in water content. The radius of the soil column was just 10 cm which is much less than the 90 cm orifice spacing which is commonly being used by the onsite industry. However, there was a tendency of better lateral distribution of wastewater in the drip versus the point application method. Therefore, field testing and side by side comparison of drip and point application systems was deemed to need further study.

2.6.2 Vertical Flow

In the literature review for this chapter, conditions for saturated flow, unsaturated flow and transient flow were described. This experiment was not conducted under steady state conditions, i.e., in conditions where the soil moisture potential remains constant and water may be flowing under saturated or unsaturated condition. Instead, it was conducted to mimic field conditions in a sewage dispersal field which is being dosed continually or intermittently each day but over many days. The TDR probe readings showed that the water content (θ) increased with depth and was close to saturation or close to tension free saturated zone (Freeze and Cherry, 1979). This could also be related to texture discontinuity in the soil column and difference in moisture retention curves of different soil layers (Table 2.1 and Figure 2.3a-c).

The TDR probe results also revealed that the water content distribution varied with depth and time (Figures 2.4-2.11) in both application systems. These data support the idea that there were a transient flow in both systems, i.e., there was a combination of saturated and unsaturated flow.

The water content in the columns receiving effluent via the drip application system was lower than the columns receiving effluent via the point application system (Figures 2.9 and 2.10). This was because, in the drip application system, wastewater was dripping in small and uniform doses while, in the point application system, there was an intermittent ponding of effluent. The differences in application methods for same volume of effluent in the columns resulted in unsaturated conditions in columns that received drip application of effluent but near saturated conditions in columns receiving effluent by the point application system (Figure 2.11).

2.7 Conclusions

The following conclusions were drawn from the experiment:

- Transient saturated flow obtained in case of point application system due to intermittent ponding.
- Transient unsaturated flow obtained due to intermittent dripping.
- Water content in the lower depth (65 cm) was higher compared to other two depths (15 cm and 35 cm).
- The water content was higher in soil received water in point application system in both horizontal depth as well as vertical depths than soil in columns received effluent in drip application system which might causes difference in bacterial transport.

2.8 References

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Chapter 3

Lateral and Vertical Distribution and Transport of Fecal Coliforms, Somatic Coliphages and Nutrients in Soil Columns Dosed with Wastewater by Point and Drip Application Methods for 60 and 90 Days

3.1 Introduction

Domestic wastewaters contain many substances such as pathogenic bacteria, infectious viruses, organic matter, toxic chemicals and excess nutrients that are undesirable and potentially harmful (Hurst et al., 1980). The presence of pathogenic bacteria in public and private water systems has emerged in the past years as a priority water quality issue (Lo et al., 2002; O'Connor, 2001). The entry of pathogenic bacteria into drinking water sources poses a great risk to human health (Craun, 1984; Jamieson et al., 2002). Therefore, wastewater must be treated in a safe and effective manner to protect the public as well as the environment (Van Cuyk et al., 2001; Anderson et al., 1991; Higgins et al., 1999; Oakley et al., 1999).

3.2 Literature Review

3.2.1 Transport of Bacteria and Viruses in Soil

Movement of microorganisms and nutrients through soil is an important process for assessing the risk of groundwater contamination. The transport of microorganisms in soil is largely related to the saturated and unsaturated flow of water (Schijven et al., 2002; Powelson and Mills, 2001; Lo et al., 2002). Stotzky (1985) found that variability in soil water content, soil texture, and soil structure influenced bacterial transport and retention mechanisms in both horizontal and vertical directions. Tan et al. (1992) attempted to quantify and model the movement of *Pseudomonas fluorescens* strain 2-79 through packed sand columns. The transport of bacteria was retarded relative to the movement of chloride and tritium probably due to adsorption since the soil pores were not thought to be small enough to strain bacteria. Transport increased in coarser sands when the soils were treated with acid because this caused a decrease in trace organic material and free iron oxides. Huysman and Verstraete (1992) also examined the influence of soil physio-chemical properties on bacterial transport in disturbed soil columns. They found that

increased bulk density and clay content decreased the migration of various *Lactobacillus* strains.

The transport of an antibiotic resistant *E. coli* strain through 280 mm deep intact and disturbed columns containing varying soil types was examined by Smith et al. (1985). Chloride solutions containing the *E. coli* strain were applied to the soil columns at rates ranging from 5 to 40 mm/h. Results revealed that all soil types became more effective at retaining bacteria when they were sieved and repacked into the columns. In intact columns, there was no relationship between soil properties (texture and bulk density) and bacterial transport. The rate of suspension (chloride solution, water and *E. coli*) application was the dominant factor affecting transport in intact columns with transport increasing as the application rate increased. Smith et al. (1985) concluded that the transport of bacteria through sieved or mixed soil columns was negligible when compared to more structured soils.

Gerba et al. (1975) reported studies in which coliform bacteria traveled from 0.6 m in fine sandy loam to 830 m in sand-gravel; bacteriophage T4 traveled up to 1600 m in a carbonate rock area. Stewart and Reneau (1981) detected migration of coliform bacteria from septic tank drain fields in both vertical and horizontal directions to monitoring wells at 152 and 305 cm depth located within 30 m of the drain fields. The extent of migration in both directions varied depending on the position of the monitoring well relative to the drain field. They attributed these differences to variations in water flow.

Somatic coliphage is proposed to be an indicator for viral contamination (Morinigo et al., 1992; and Havelaar et al., 1993, Kouznetsov, et al., 2004) and fecal coliform is a common indicator for bacterial contamination. Juma et al. (2007) only measured fecal and total coliforms in at-grade dispersal fields. Measurement and transport of somatic coliphages in these fields could significantly contribute to our knowledge and understanding of the transport of viruses through the soil.

3.2.2 Transport of Protozoa in Soil

It was generally believed that movement of pathogens through the soil is minimal, however Mawdsley et al. (1996) have shown that appreciable numbers of *Cryptosporidium parvum* oocysts (protozoa) may be transported via preferential or

fingered flow to groundwater. Studies of packed columns with saturated flow by Brush et al. (1999) and Harter et al. (2000) and undisturbed columns with unsaturated flow (Mawdsley et al., 1996) showed that *C. parvum* oocysts could be transported rapidly downward through the soil. Although transport of *C. parvum* oocysts in saturated flow has been studied experimentally and described mathematically (Brush et al., 1999; Harter et al., 2000), detailed observations of the transport and persistence of *C. parvum* oocysts in unsaturated soils with preferential flow are still lacking, particularly in the presence of preferential flow processes. Consequently, one would expect that the modeling retention of *C. parvum* oocysts under saturated and unsaturated conditions would yield different patterns.

3.2.3 Die-Off of Bacteria in Soils

The availability of enteric bacteria for transport in runoff and leachate during precipitation events is largely influenced by the die-off rate of fecal coliform in the soil-waste system (Reddy et al. 1981). A wealth of information has been produced within the past 30 years on the survival of various enteric bacterial species in soil and groundwater systems. A review presented by Gerba et al. (1975) reported that survival times of enteric bacteria in soil and groundwater ranged from 2 to 4 months. Filip et al. (1988) examined the survivability of several organisms in simulated conditions of saturated soil and observed that most organisms tested for, including *E. coli*, survived for over 100 days at 10°C. Kudva et al. (1998) found that *E. coli* O157:H7 survived for 630 days in sheep manure that was not aerated and stored at air temperatures below 23 °C. Entry et al. (2000a, 2000b) monitored concentrations of fecal coliform (FC) bacteria in soil and runoff water from grassed buffer strips that had received liquid swine waste. After 90 to120 days, FC levels were not significantly different from strips that had not received waste. Reddy et al. (1981) conducted a review of bacterial survival and attempted to develop first order rate constants to describe the die-off of several indicator organisms and pathogens in soil systems. Average first order die-off rate constants were 1.14 d^{-1} for FC and 0.41 d^{-1} for FS (fecal Streptococci). Average rate constants for specific pathogens were 1.33 d^{-1} for *Salmonella* and 0.68 d^{-1} for *Shigella*. Sjogren (1994) assessed the survival of *E. coli* and used exponential regression to estimate survival times in soil.

Survival times were estimated by extrapolating the die-off curve to zero counts of bacteria. Probable survival times ranged from 20.7 to 23.3 months.

3.2.4 Point versus Drip Application Methods

Point and drip application of effluent are the two acceptable methods for wastewater effluent application to soil with onsite systems. Drip dispersal is a new technology which could be used as an alternative to the conventional perforated drainage pipe. It is becoming more popular because the wastewater is applied in frequent, small uniform doses, allowing the soil system more time to treat the wastewater before it reaches the groundwater, even on less suitable soil types (Bohrer and Converse, 2001). The application of wastewater to the surface or subsurface through drip irrigation also showed a high degree of microorganism decay through die-off and predation (Reddy et al. 1981) . A higher percentage of fecal bacteria died when wastewater was applied through subsurface drip irrigation system from a stabilization pond system (Compos et al., 2000) rather than in an at-grade system. So, more studies are needed to quantify the dynamics of bacteria and viruses in soil specially their movement in saturated and unsaturated flow in vertical and in lateral soil layers.

3.3 Objective

The objective of this research was to quantify the lateral and vertical distribution and transport of fecal coliforms, somatic coliphages and nutrients (NH_4-N, NO_3-N and Kelowna extractable P) in soil columns dosed with wastewater by point and drip application methods for 60 and 90 days

3.4 Material and Methods

3.4.1 Sampling Concept

The first set of 4 columns was sacrificed after 60 days and the second set was sacrificed after 90 days of daily effluent applications. The 144 soil samples from 4 columns for each sampling date [2 soil columns x 2 application methods x 6 depths x 3 lateral layers for each depth x 2 (duplicate) analysis] were analyzed for NH_4^+, NO_3^-, and

Kelowna extractable PO_4^{3-}. Another 144 samples (72 samples in duplicate) were also collected for the analysis of fecal coliforms and somatic coliphages.

3.4.2 Soil Sampling Procedure

A schematic diagram for sampling sections of the soil column is presented in Fig. 3.1

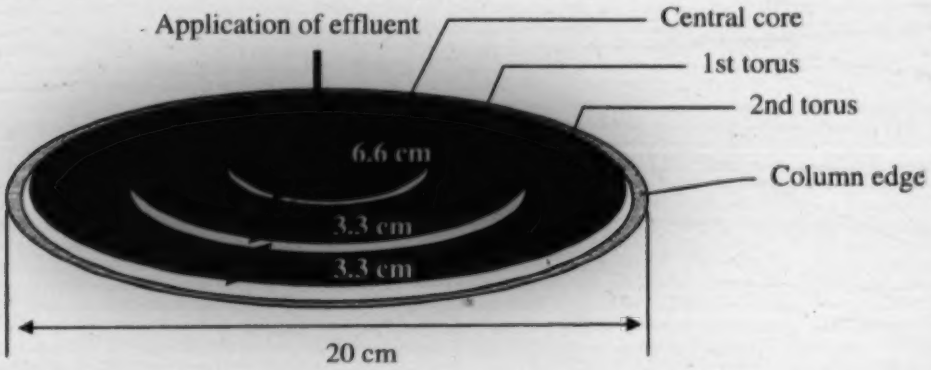

Figure 3.1. Soil sampling technique of the experimental soil column

When the columns were sacrificed for sampling, they were laid horizontally (Photo 3.1a) and the soil column was then dissected at the precut, glued depths (Photo 3.1b). The cuts were made at 20, 50 and 75 cm depths.

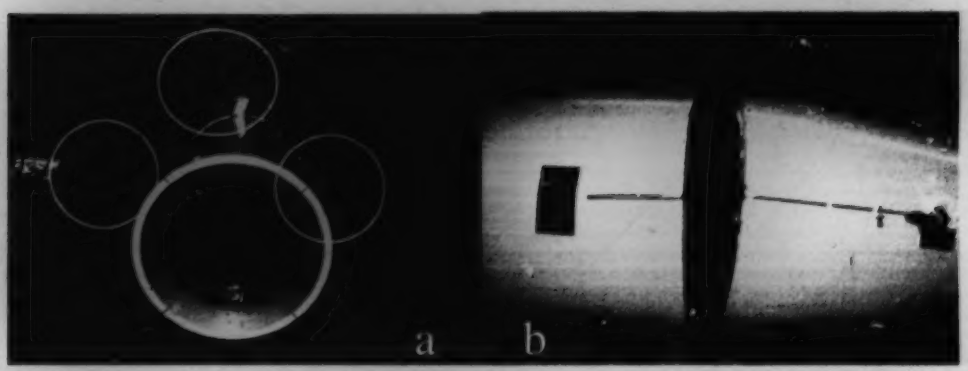

Photo 3.1. Photographs showing dissection of soil columns (a) and separation of soil column sections (b).

The sampling sequence was from the bottom to top soil layers and from second torus to the central core for each layer. The soil from the two top layers was divided into two depths. Thus soil samples were obtained for 0-10, 10-20, 20-35, 35-50, 50-75 and 75-100 cm depths. The procedure is outline below.

For each layer, two 30 cm long concentric steel cylinders, 66 and 132 mm diameters were used to sample the soil in the PVC columns (200 mm diameter). A cardboard torus (a donut-shaped object), 198 mm wide and 66 mm inside diameter, was first centered inside the cylinder. This permitted the placement of the smaller sampling cylinder (66 mm diameter) in the center of the PVC column. The cylinder was pushed downwards to a 20 cm depth. This cylinder contained the soil from the central core of the column. The cardboard torus was then removed.

Then another cardboard torus, 198 mm wide and 132 mm inside diameter, was placed in between the PVC pipe and small steel cylinder. The second, larger sampling cylinder (132 mm diameter) was centered in the PVC column and pushed downwards to 20 cm depth. The soil sample between the two steel cylinders was the soil from the first torus.

Photo 3.2. Column pieces and inserted steel cylinders with two cardboard torus. The Letter A represents the small steel cylinder, B represents the larger steel cylinder and letter C represents the PVC pipe.

Then the PVC pipe was removed and the soil between the PVC pipe and the second sampling cylinder was collected (Photo 3.3a). This sample was the soil from the second torus. The soil samples were collected first from the second torus. Then the second larger steel sampling cylinder was removed which exposed the soil from the first torus (Photo

3.3b) which was sampled. Finally, the soil was scraped from the small cylinder. The steel cylinders were sterilized every time before being used for different depths.

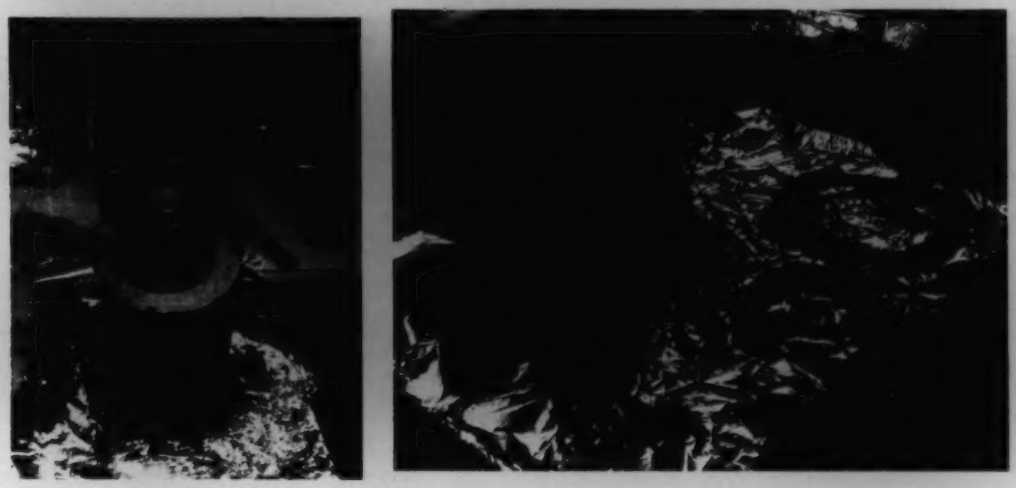

Photo 3.3. Soil from the second torus was exposed after removal of PVC pipe is shown on the left and soil from the central core and first torus is shown on the right. The cardboard tori are also shown in the picture.

The soil was sampled at 0-10, 10-20, 20-35, 35-50, 50-75 and 75-100 cm depths from the respective sections in the PVC column. A total of 36 samples were collected from each column that corresponded to three lateral locations (Figure 3.1 and Photo 3.2) and six vertical layers with 2 replications. Data from these samples were used to assess the lateral and vertical distribution and transport of wastewater, nutrients, fecal coliforms and coliphages through the soil column.

3.4.3 Chemical Analysis:

The pH of the soil samples was determined in 1:2.5 soil: water suspension and electrical conductivity (EC) was determined in the supernatant liquid of 1:1 soil: water using an Accumet Research AR20 pH/conductivity meter (Fisher Scientific, Model No. S/N AR 93316577) to characterize the soil. NO_3-N and NH_4-N of the soil samples were extracted with 2M KCl and then analyzed by Ion chromatography (Westco SmartChem 200, Westco Scientific, USA).

Acid soluble phosphorus was determined by extracting with Kelowna extractant. The Kelowna extracting solutions were prepared by dissolving 77.08 g/L ammonium acetate, 0.543 g/L ammonium fluoride and 28.74 ml/L glacial acetic acid to make a solution of 0.015M NH_4F, 1M NH_4OAC in 0.5M CH_3COOH. The soil samples were extracted with Kelowna solution and were analyzed for PO_4 by Ion Chromatography in the Department of Renewable Resources Analytical Laboratory located in the Earth Sciences Building, University of Alberta Campus.

3.4.4 Microbiological Analysis:

3.4.4.1 Enumeration of Fecal Coliforms

Turco (1994) recommended using the Multiple Tube Fermentation (MTF) method to determine total and fecal coliform populations in the soil. The Membrane Filtration (MF) method, used to enumerate coliforms in water, was not used because the particles can clog the filter and interfere with the test results. For these reasons, MTF was used to determine the numbers of coliform bacteria in soil samples as well as primary effluent (PE) and diluted primary effluent (DPE).

Briefly, MTF uses Most Probable Numbers (MPN) to determine the number of fecal coliforms (FC) in soil and waste water samples. Serial dilutions of samples are added to test tubes containing Lauryl Tryptose Broth and a small inverted tube (Durham tube) is used to help detect gas bubbles. The test tubes are incubated at 35^0C for 24h. Bacterial growth and gas formation indicate the presence of presumed total coliforms. Positive tubes are then re-tested using EC media to determine the presence of fecal coliforms. Numbers of positive tubes are used in conjunction with MPN, a statistical method based on the random dispersion of bacteria in a given sample, to calculate the number of fecal coliforms in a sample. A more detailed description of the lab procedure used in this study is given in Juma et al. (2007).

3.4.4.2 Enumeration of Somatic Coliphages

Somatic coliphages were quantified by the standard procedure (APHA, 2005) using *E. coli* CN (ATCC 600709) strain. Nalidixic acid (Sigma N4382) antibiotic solution was used to inhibit growth of other bacteria. The Single Agar Layer (SAL) assay was used

which is based on the plaque assay. In this method 100 ml sample volumes are assayed in 150 mm assay dishes and the results are expressed as plaque forming unit per 100 ml (pfu/100 ml) for the leachates, PE, and DPE and as (pfu/g soil) for soil samples.

Photo 3.4. Photograph of (a) fecal coliforms (b) and somatic coliphages determined in the experiment

3.4.5 Leachate collection:

Leachate was collected in sterilized glass containers from each column every day, pooled and analyzed on Wednesday and Saturday during the experiment. Leachates were analyzed for NH_4^+, NO_3, and extractable PO_4^{3-}, fecal coliforms and somatic coliphages by standard methods as described above.

3.5. Results

3.5.1 Nutrients and Organisms in Leachates:

3.5.1.1. NH_4-N content:

The content of NH_4 in leachate in 60 and 90 days after effluent application is shown in the Table 3.1. The NH_4 content in the leachate did not differ significantly among the treatments and with the duration (days) of the experiment. But it differed significantly in leachate from one week to another. The interaction of treatment and week was found to be significant (P=0.0047) for NH_4^+ in the leachates (Table 3.1).

Table 3.1. Content of NH_4-N (ppm) in leachate during 60 and 90 days of effluent application. The values are mean of two replicate columns with ± standard deviations.

Week	NH_4-N (ppm)			
	P-60	D-60	P-90	D-90
2	0.19±0.05	0.15±0.03	0.20±0.01	0.16±0.03
3	0.16±0.01	0.12±0.04	0.18±0.02	0.14±0.02
4	0.14±0.09	0.16±0.01	0.16±0.03	0.18±0.01
5	0.16±0.05	0.15±0.02	0.16±0.01	0.14±0.03
6	0.15±0.01	0.14±0.05	0.16±0.05	0.12±0.04
7	0.14±0.02	0.16±0.02	0.14±0.02	0.17±0.03
8	0.16±0.03	0.12±0.01	0.14±0.06	0.11±0.02
9	0.12±0.02	0.16±0.03	0.10±0.01	0.18±0.05
10	-	-	0.12±0.01	0.18±0.03
11	-	-	0.11±0.02	0.14±0.02
12	-	-	0.15±0.03	0.18±0.02
13	-	-	0.16±0.02	0.18±0.03

Summary of ANOVA

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.0596	NS
Days	0.2651	NS
Treatment* Days	0.1097	NS
Week (Days)	0.0032	Significant
Treatment*Week (Days)	0.0047	Significant

Before adding the effluent to the columns, distilled water was added for one week to observe water movement through the column and the collected leachates were not analyzed. The effluent was added for one week to displace the distilled water from the columns and the leachates also were not analyzed. In the second week, NH_4-N concentration was the highest in the P-90 compared to other treatments. On the 4th week of effluent application, D-90 had the highest concentration of NH_4-N in the leachate compared to other treatments. On the 8th week, P-60 had the highest concentration of NH_4-N in the leachate compared to other treatments. Although the concentrations varied, NH_4-N was being collected in the leachates. The P-60 and P-90 results vary from each other which would be due to the effect of mean values of the columns.

3.5.1.2. NO_3-N content:

All treatment and interaction effects on NO_3-N in leachate during 60 and 90 days of effluent application were insignificant. Quantitatively, the NO_3-N was greater than NH_4-N which was being collected in the leachates (Table 3.2).

Table 3.2. Content of NO_3-N (ppm) in leachate during 60 and 90 days of effluent application. The values are mean of two replicate columns with ± standard deviations.

Week	NO_3-N (ppm)			
	P-60	D-60	P-90	D-90
2	2.18±0.05	2.47±0.13	4.2±0.10	3.27±0.09
3	2.18±0.07.	1.83±0.07	3.3±0.16	4.68±0.12
4	0.96±0.04	2.97±0.23	2.4±0.15	2.89±0.11
5	4.10±0.15	1.70±0.06	2.32±0.9	3.33±0.17
6	0.31±0.06	0.20±0.02	0.18±0.05	0.21±0.09
7	4.09±0.20	3.46±0.05	0.18±0.03	3.42±0.08
8	0.20±0.05	3.20±0.09	3.24±0.16	2.12±0.13
9	3.18±0.16	2.22±0.06	4.50±0.50	2.20±0.10
10	-	-	0.22±0.08	4.27±0.13
11	-	-	0.50±0.05	3.10±0.15
12	-	-	1.70±0.11	2.20±0.08
13	-	-	4.25±0.50	4.51±0.49

Summary of ANOVA

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.160	NS
Days	0.853	NS
Treatment* Days	0.605	NS
Week(Days)	0.291	NS
Treatment*Week(Days)	0.712	NS

3.5.1.3. Water soluble PO_4:

All treatment and interaction effects on PO_4 in leachate during 60 and 90 days of effluent application were insignificant. However, small quantities of inorganic PO_4 were being collected in the leachates (Table 3.3).

Table 3.3. Content of inorganic PO_4 (ppm) in leachate during 60 and 90 days of effluent application. The values are mean of two replicate columns with ± standard deviations.

Week	PO_4 (ppm)			
	P-60	D-60	P-90	D-90
2	0.19±0.06	0.17±0.02	0.18±0.03	0.18±0.04
3	0.15±0.02	0.14±0.03	0.18±0.04	0.14±0.06
4	0.15±0.05	0.15±0.05	0.11±0.05	0.12±0.02
5	0.10±0.04	0.05±0.02	0.12±0.04	0.07±0.05
6	0.11±0.07	0.14±0.04	0.11±0.05	0.15±0.03
7	0.17±0.04	0.17±0.03	0.22±0.04	0.14±0.02
8	0.18±0.04	0.15±0.02	0.12±0.02	0.17±0.05
9	0.15±0.07	0.11±0.03	0.13±0.05	0.10±0.02
10	-	-	0.12±0.04	0.15±0.03
11	-	-	0.15±0.03	0.21±0.05
12	-	-	0.11±0.01	0.18±0.01
13	-	-	0.12±0.02	0.15±0.03

Summary of ANOVA

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.111	NS
Days	0.504	NS
Treatment* Days	0.413	NS
Week(Days)	0.589	NS
Treatment*Week(Days)	0.367	NS

3.5.1.4. Fecal coliforms:

Fecal coliforms were not found in the leachate during the experimental periods of 60 and 90 days. Thus, the fecal coliforms were being attenuated or inactivated in the soil columns.

3.5.1.5. Somatic coliphages:

Somatic coliphages were found (5-18 pfu/100 ml) in the leachate during the experiment time but concentration of the somatic coliphages were much higher (80 pfu/100 ml) in the applied effluent. In average 78 – 94%, somatic coliphages were removed while passing through the soil column. The treatment, days and week were found non-significant for somatic coliphages in leachate. The content of somatic coliphages in leachate in 60 and 90 days after effluent application is shown in the Table 3.4.

Table 3.4. Number of somatic coliphages in leachate during 60 and 90 days of effluent application. The values are mean of two replicate columns with ± standard deviations.

Week	Number of somatic coliphages (pfu/100 ml)			
	P-60	D-60	P-90	D-90
2	10±0.5	6±0.8	12±0.25	9±0.50
3	12±1.0	8±1.0	12±0.50	10±2.13
4	11±2.0	8±0.6	13±0.80	8±1.50
5	17±3.0	11±1.0	14±0.60	11±2.60
6	18±2.0	12±0.75	17±1.87	13±1.10
7	16±0.5	8±0.21	16±1.16	10±1.45
8	15±2.5	14±0.45	14±1.30	15±0.80
9	15±0.25	10±0.91	18±2.50	11±2.10
10	-	-	11±1.25	18±2.58
11	-	-	12±1.54	6±1.16
12	-	-	13±2.11	13±2.09
13	-	-	9±1.28	8±1.80

Summary of ANOVA

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.170	NS
Days	0.872	NS
Treatment* Days	0.560	NS
Week(Days)	0.292	NS
Treatment*Week(Days)	0.721	NS

3.5.2 Results of soil analysis:

3.5.2.1. Gravimetric water content:

ANOVA showed that gravimetric moisture content of the soil did not differ significantly among the treatments, duration of the experiment and lateral layers. All interactions were also not significant. Only soil depth had a significant effect on the gravimetric moisture content (Table 3.5).

Table 3.5. Probability of F values from Analysis of Variance of gravimetric moisture content (%) in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.304	NS
Days	0.187	NS
Treatment* Days	0.349	NS
Depth	<0.001	Significant
Depth* Treatment	0.372	NS
Depth*Days	0.054	NS
Treatment*Depth*Days	0.773	NS
Lateral layer	0.705	NS
Treatment* Lateral layer	0.885	NS
Days* Lateral layer	0.464	NS
Treatment*Days* Lateral layer	0.120	NS
Depth* Lateral layer	0.819	NS
Treatment*Depth* Lateral layer	0.786	NS
Depth*Days* Lateral layer	0.871	NS
Treatment*Depth*Days*Lateral layer	0.673	NS

In order to analyze the data for depth effect in greater detail, the means for moisture content by depth and lateral layers were analyzed with a Duncan's multiple range test for four treatments: Method (Point, Drip) x Duration (60, 90 days). The data for the P-60 are presented in Figure 3.2 and Table 3.6. The soil moisture content in the central core at the 0-10, 10-20 and 35-50 cm intervals was significantly higher than that in the second torus. As the column was being dosed daily, the moisture content was the highest in the surface horizons. Moisture content was also high in the lowest soil layer in the columns because it had a clay texture. In P-90, the moisture content in the central core was higher than the second torus at all the depths except at 35-50 and 75-100 cm (Figure 3.3 and Table 3.7). The dominant flow of moisture was downward in all these soil columns.

There was no difference between in the soil gravimetric moisture content in the lateral layers at all depths except for 35-50 cm in D-60 and 35-50 and 50-75 depths in D-90 (Figures 3.4 and 3.5; Tables 3.8 and 3.9). These results showed there were remarkable differences in soil moisture content in soil columns due to the method of application of the effluent.

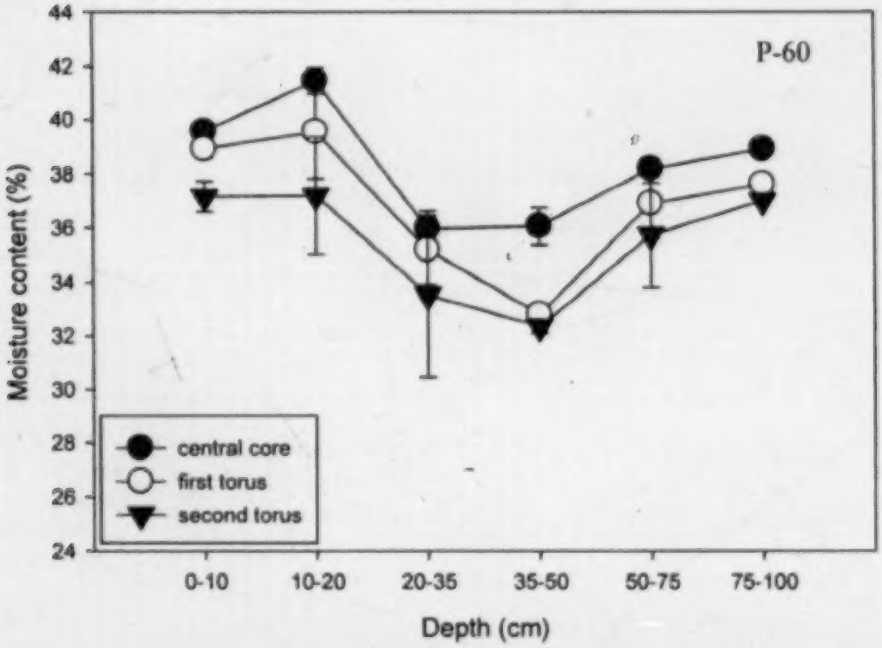

Figure 3.2. Gravimetric moisture (dry basis) content in different depths and lateral layers of soil column at Day 60 with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.6. Gravimetric moisture (dry basis) content (%) in different depths and lateral layers of soil column with point application of effluent at Day 60. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth (cm)	Central core		First torus		Second torus	
0-10	**39.6**	**ab**	**38.9**	**abc**	**37.2**	**cde**
10-20	**41.5**	**a**	**39.6**	**ab**	**37.2**	**de**
20-35	36.0	def	35.2	efg	33.5	fgh
35-50	**36.1**	**cdef**	**32.8**	**gh**	**32.4**	**h**
55-75	38.2	bcd	36.9	bcde	35.7	def
75-100	38.9	abc	37.6	bcde	37.0	bcde

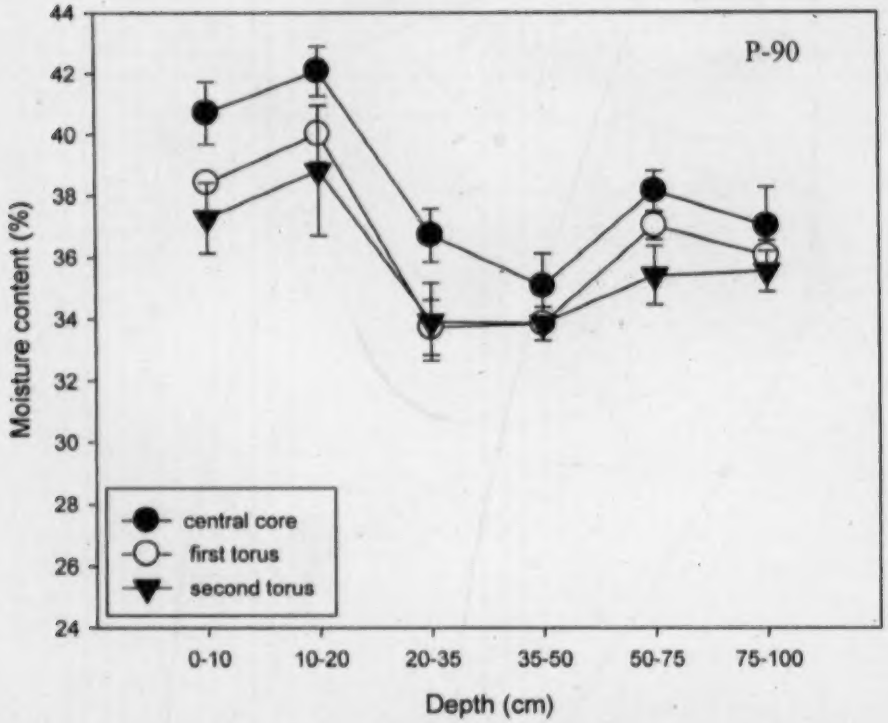

Figure 3.3. Gravimetric moisture (dry basis) content in different depths and lateral layers of soil column at Day 90 with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.7. Gravimetric moisture (dry basis) content (%) in different depths and lateral layers of soil column with point application of effluent at Day 90. The values are mean of two replicates. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth (cm)	Central core	First torus	Second torus
0-10	**40.7 ab**	**38.4 cd**	**37.3 def**
10-20	**42.1 a**	**40.0 abc**	**38.8 bcd**
20-35	**36.7 def**	**33.7 h**	**33.9 gh**
35-50	35.1 fgh	33.9 gh	33.9 gh
55-75	**38.2 cde**	**37.0 def**	**35.4 fgh**
75-100	37.0 def	36.1 efg	35.6 fgh

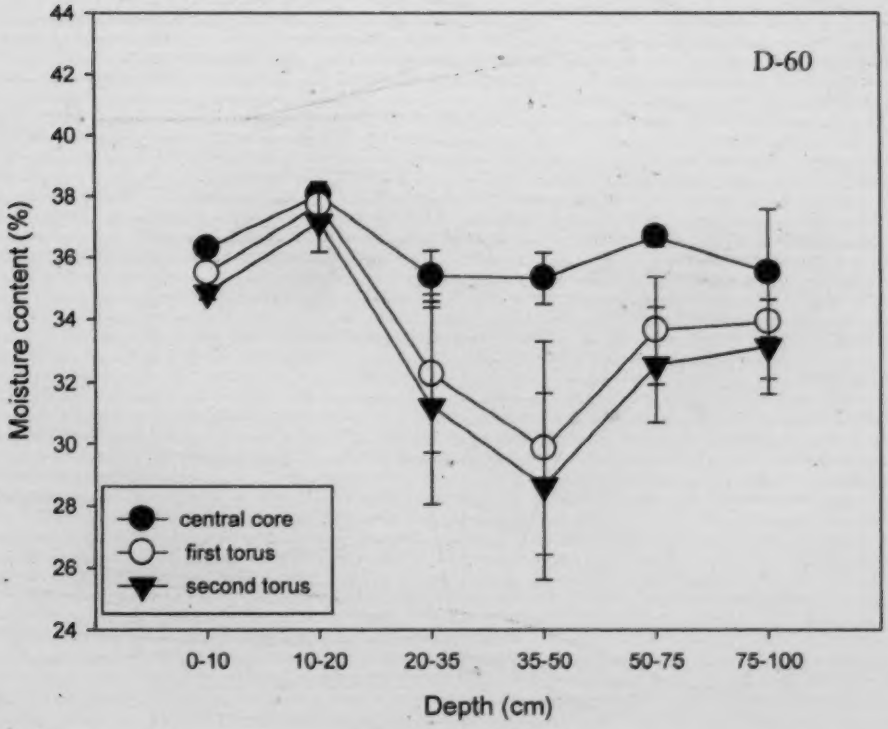

Figure 3.4. Gravimetric moisture (dry basis) content in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.8. Gravimetric moisture (dry basis) content (%) in different depths and lateral layers of soil column with drip application of effluent at Day 60. The values are mean of two replicates. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth (cm)	Central core	First torus	Second torus
0-10	36.3 ab	35.5 abc	34.8 abc
10-20	38.0 a	37.7 a	37.1 a
20-35	35.4 abc	32.3 abcd	31.2 bcd
35-50	**35.3 abc**	**29.9 cd**	**28.6 d**
55-75	36.5 ab	33.6 abcd	32.5 abcd
75-100	35.5 abc	33.9 abcd	33.1 abcd

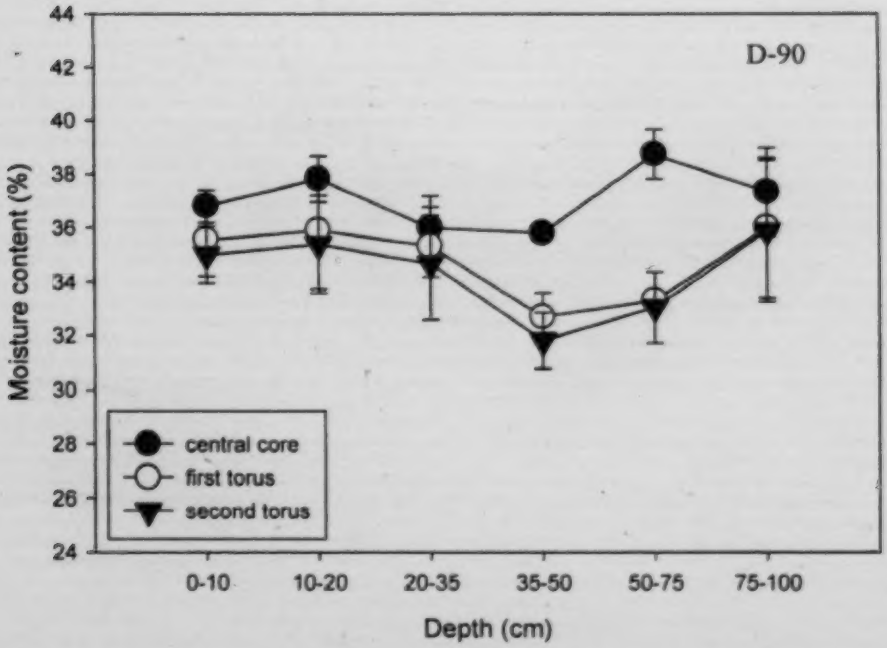

Figure 3.5. Gravimetric moisture (dry basis) content (%) in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.9. Gravimetric moisture (dry basis) content (%) in different depths and lateral layers of soil column with drip application of effluent for Day 90. The values are mean of two replicates. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth (cm)	Central core	First torus	Second torus
0-10	36.8 abc	35.6 abcd	35.0 bcde
10-20	37.8 ab	35.9 abcd	35.4 abcd
20-35	36.0 abcd	35.3 abcde	34.7 bcde
35-50	**35.8 abcd**	**32.7de**	**31.8 e**
55-75	**38.7 a**	**33.3 cde**	**33 de**
75-100	37.3 ab	36 abcd	36 abcd

3.5.2.2 Ammonium-Nitrogen content:

The NH_4-N content in the soil did not differ significantly among the treatments and with the duration (days) of the experiment but it decreased significantly with vertical depth (P=0.0001) and lateral layers (P=0.0001). The depth*lateral layers interaction was significant (Table 3.10).

Table 3.10. Probability of F values from Analysis of Variance of NH_4-N in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.117	NS
Days	0.518	NS
Treatment* Days	0.052	NS
Depth	0.0001	Significant
Depth* Treatment	0.043	NS
Depth*Days	0.235	NS
Treatment*Depth*Days	0.023	NS
Lateral layers	0.0001	Significant
Treatment* Lateral layers	0.186	NS
Days* Lateral layers	0.178	NS
Treatment*Days* Lateral layers	0.552	NS
Depth* Lateral layers	0.0001	Significant
Treatment*Depth* Lateral layers	0.103	NS
Depth*Days* Lateral layers	0.405	NS
Treatment*Depth*Days*Lateral layers	0.056	NS

In P-60, the NH_4-N in the central core at the 10-20 and 35-50 cm depths was significantly higher that in the second torus (Figure 3.6, Table 3.11) however this difference was only significant for the 10-20 cm depth in P-90 (Figure 3.7, Table 3.12). In D-60, these differences were significant for 0-10 and 10-20 cm depths (Figure 3.8, Table 3.13) however these were only significant for the 10-20 cm depth in D-90 (Figure 3.9, Table 3.14). There were significant decreases in NH_4-N with depth under each lateral layer in both treatments.

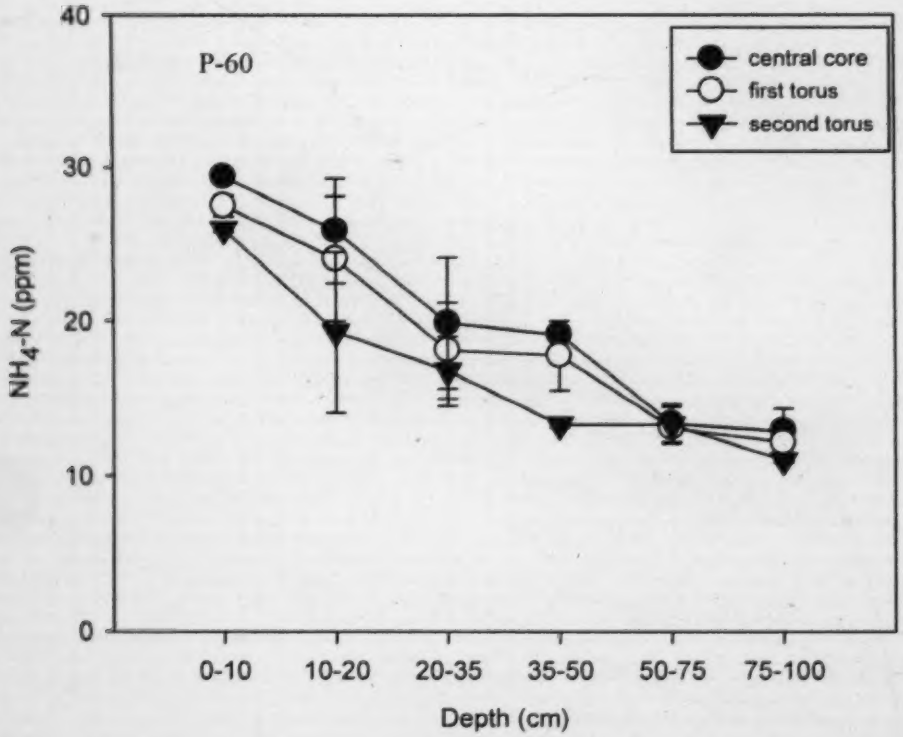

Figure 3.6. NH_4-N at Day 60 in different depths and lateral layers of soil column with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.11. The NH_4-N content at Day 60 in three lateral layers of the six different vertical depths in point application system for 60 days. The values are mean of two replicates. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NH_4-N (ppm)		
	Central core	1st torus	2nd torus
0-10	29.4 a	27.5 a	26.0 a
10-20	**25.9 a**	**24.0 ab**	**19.3 bc**
20-35	19.8 bc	18.1 cd	16.7 cde
35-50	**19.1 bc**	**17.7 cde**	**13.3 def**
50-75	13.3 def	13.1 def	13.3 def
75-100	12.9 def	12.1 ef	11.0 f

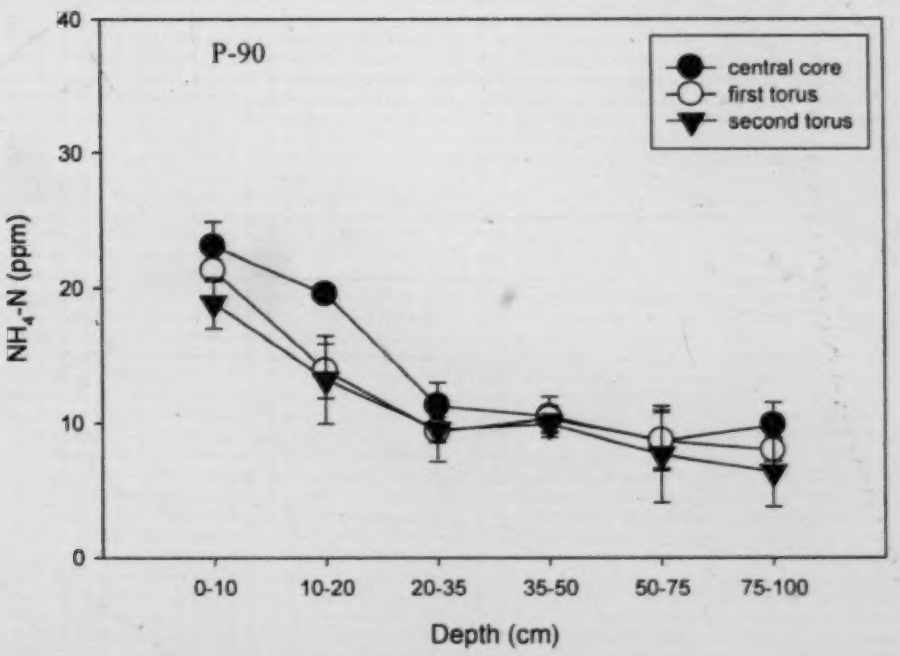

Figure 3.7. NH_4-N at Day 90 in different depths and lateral layers of soil column with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.12. The NH_4-N content at Day 90 in three lateral layers of the six different vertical depths in point application system for 90 days. The values are mean of two replicates. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NH_4-N (ppm)		
	Central core	First torus	Second torus
0-10	23.0 a	21.2 a	18.8 a
10-20	**19.5 a**	**13.8 b**	**13.1 bc**
20-35	11.2 bcd	9.3 bcde	9.5 bcde
35-50	10.4 bcde	10.3 bcde	9.9 bcde
50-75	8.7 cde	8.6 cde	7.6 de
75-100	9.8 bcde	7.9 de	6.3 e

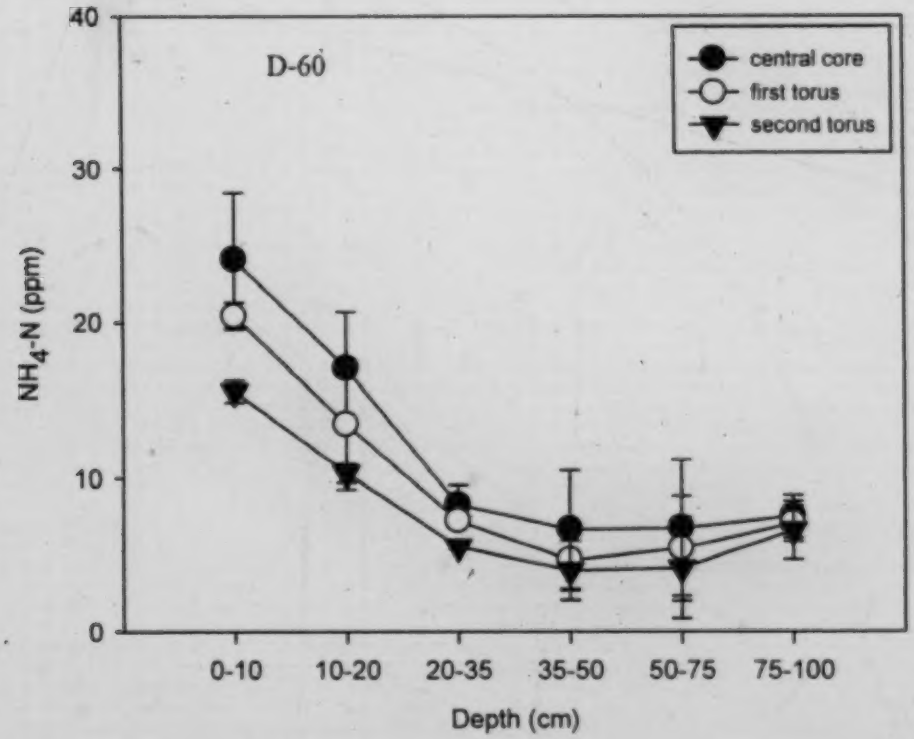

Figure 3.8. NH_4-N in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.13. The NH_4-N at Day 60 in three lateral layers of the six different vertical depths in drip application system for 60 days. The values are mean of two replicate columns. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NH_4-N (ppm)		
	Central core	First torus	Second torus
0-10	**24.0 a**	**20.4 ab**	**15.6 bcd**
10-20	**17.0 bc**	**13.4 cde**	**10.3 def**
20-35	8.2 ef	7.2 f	5.5 f
35-50	6.6 f	4.7 f	4.0 f
50-75	6.7 f	5.4 f	4.1 f
75-100	7.5 ef	7.0 f	6.6 f

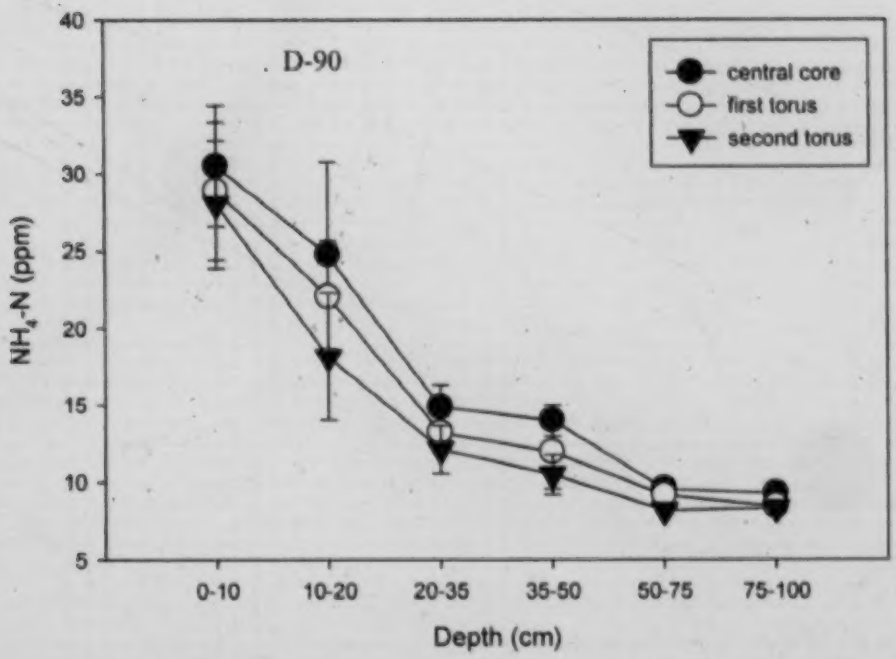

Figure 3.9. NH_4-N in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.14. The NH_4-N at Day 90 in three lateral layers of the six different vertical depths in drip application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NH_4-N (ppm)		
	Central core	First torus	Second torus
0-10	30.5 a	28.8 a	28.0 ab
10-20	**24.7 ab**	**22.0 bc**	**18.1 cd**
20-35	14.8 de	13.2 def	12.1 def
35-50	13.9 def	11.9 def	10.4 ef
50-75	9.5 ef	9.1 ef	8.2 ef
75-100	9.2 ef	8.4 ef	8.3 ef

3.5.2.3 Nitrate-Nitrogen content:

The NO_3-N content in the soil did not differ significantly among the treatments nor with the duration (days) of the experiment but it decreased significantly with vertical depth and lateral layers. The depth*lateral layers interaction was significant (Table 3.15).

Table 3.15. Probability of F values from Analysis of Variance of NO_3-N in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significant at 95% confidence level
Treatment	0.489	NS
Days	0.785	NS
Treatment* Days	0.175	NS
Depth	0.0001	Significant
Depth* Treatment	0.122	NS
Depth*Days	0.705	NS
Treatment*Depth*Days	0.870	NS
Lateral layers	<0.001	Significant
Treatment* Lateral layers	0.262	NS
Days* Lateral layers	0.134	NS
Treatment*Days* Lateral layers	0.062	NS
Depth* Lateral layers	0.001	Significant
Treatment*Depth* Lateral layers	0.228	NS
Depth*Days* Lateral layers	0.080	NS
Treatment*Depth*Days*Lateral layers	0.585	NS

There were no significant differences in NO_3-N between lateral layers at all depths and treatments except for D-60 where the NO_3-N in the central core at the 10-20 cm depth was significantly higher that in the second torus (Figures 3.10-13, Tables 3.16-19). This difference disappeared by Day 90. There were significant decreases in NO_3-N with depth under each lateral layer in both treatments.

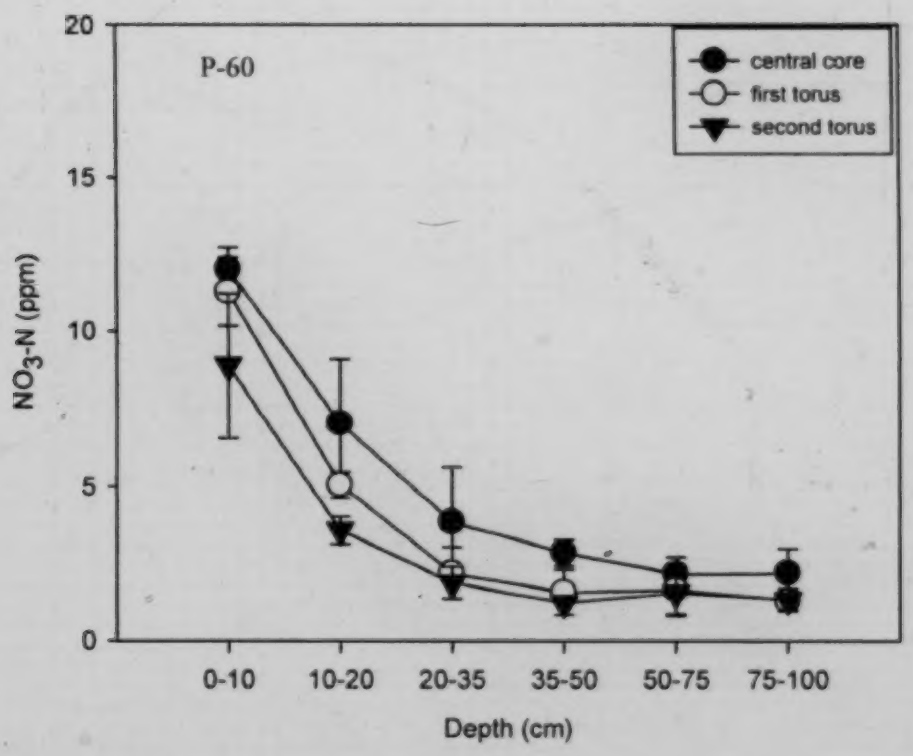

Figure 3.10. NO_3-N in different depths and lateral layers of soil column at Day 60 with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.16. The NO_3-N content in three lateral layers of the six different vertical depths in point application system for 60 days. The values are mean of two replicate columns. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NO_3-N (ppm)		
	Central core	First torus	Second torus
0-10	12.0 a	11.2 a	8.9 b
10-20	7.0 bc	5.0 cd	3.5 def
20-35	3.8 de	2.2 ef	1.8 ef
35-50	2.8 def	1.5 ef	1.2 f
50-75	2.1 ef	1.6 ef	1.5 ef
75-100	2.2 ef	1.3 f	1.3 f

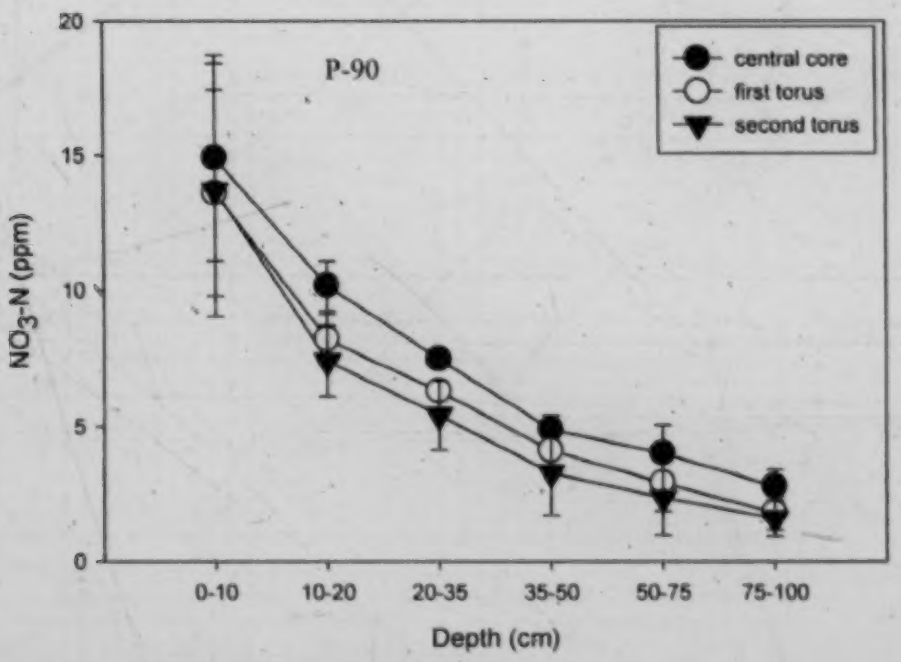

Figure 3.11. NO_3-N in different depths and lateral layers of soil column at Day 90 with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.17. The NO_3-N content in three lateral layers of the six different vertical depths in point application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	NO_3-N (ppm)		
	Central core	First torus	Second torus
0-10	14.9 a	13.6 ab	13.7 ab
10-20	10.1 bc	8.2 cd	7.4 cde
20-35	7.4 cde	6.3 cdef	5.4 cdef
35-50	4.8 def	4.1 def	3.2 ef
50-75	4.0 def	2.9 ef	2.3 f
75-100	2.7 ef	1.7 f	1.5 f

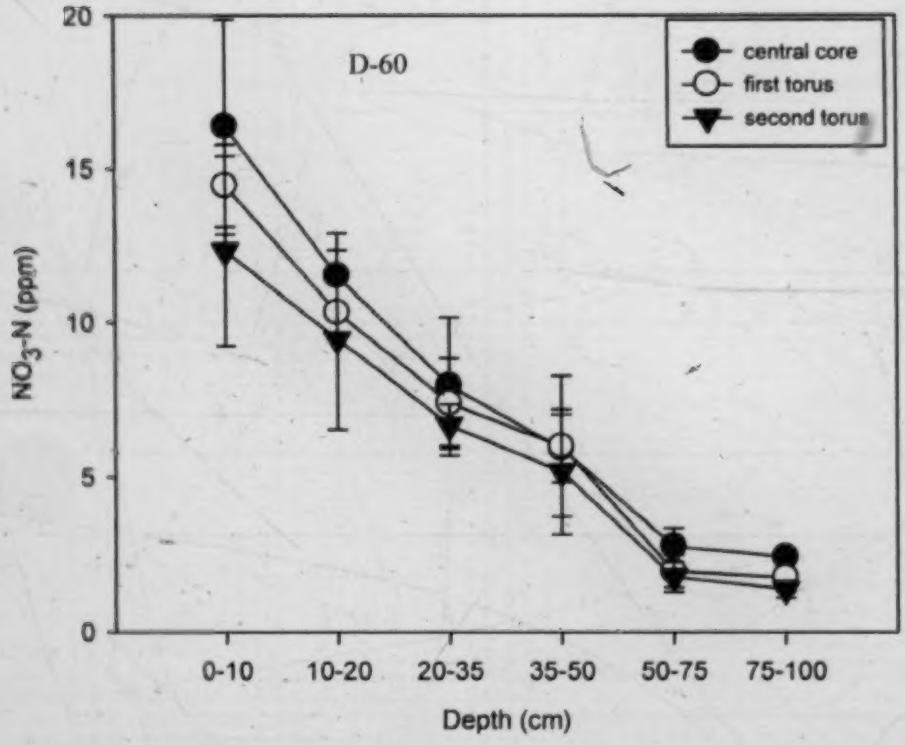

Figure 3.12. NO_3-N in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.18. The NO_3-N content at Day 60 in three lateral layers of the six different vertical depths in drip application system for 60 days. The values are mean of two replicates. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Lateral layers, NO_3-N (ppm)		
	Central core	First torus	Second torus
0-10	**16.3 a**	**14.4 ab**	**12.3 bc**
10-20	11.5 bcd	10.3 cde	9.4 cdef
20-35	7.9 defg	7.3 efg	6.6 efgh
35-50	5.9 fghi	6.0 fghi	5.1 ghij
50-75	2.7 hij	1.9 ij	1.8 j
75-100	2.4 ij	1.7 j	1.3 j

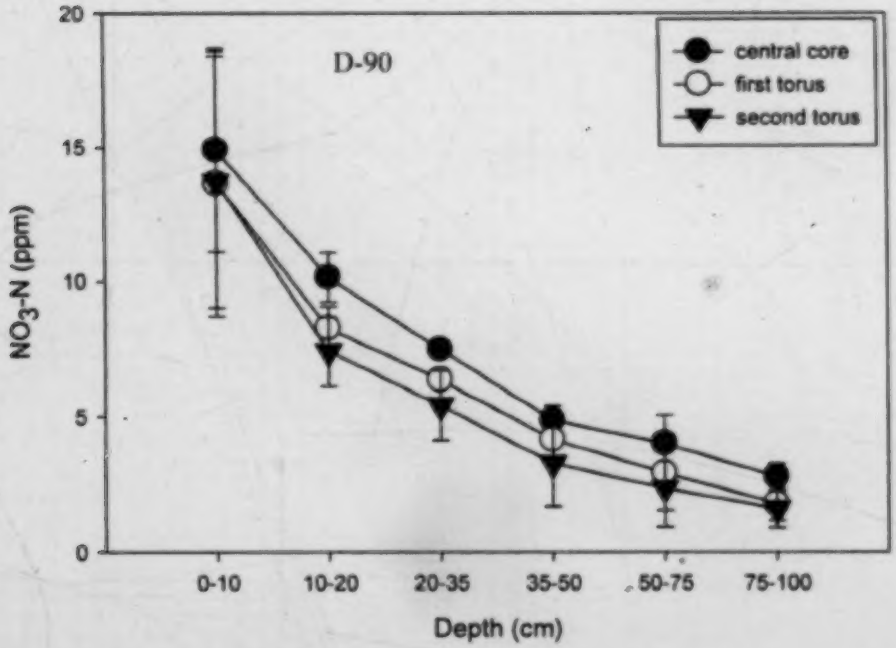

Figure 3.13. NO_3-N in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.19. The NO_3-N content in three lateral layers of the six different vertical depths in drip application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Lateral layers, NO_3-N (ppm)		
	Central core	First torus	Second torus
0-10	14.9 a	13.6 ab	13.7 ab
10-20	10.1 bc	8.2 cd	7.4 cde
20-35	7.4 cde	6.3 cdef	5.4 cdef
35-50	4.8 def	4.1 def	3.2 ef
50-75	4.0 def	2.9 ef	2.3 f
75-100	2.7 ef	1.7 f	1.5 f

3.5.2.4 Kelowna Extractable Phosphorus:

There were many significant effects and interactions for Kelowna-P content in the soil columns (Table 3.20). The interactions between treatment and depth, and treatment and lateral layers will be examined through graphs and Duncan's multiple range test comparisons.

Table 3.20. Probability of F values from Analysis of Variance of Kelowna-P in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significant at 95% confidence level
Treatment	<0.001	Significant
Days	<0.001	Significant
Treatment* Days	<0.001	Significant
Depth	<0.001	Significant
Depth* Treatment	0.003	Significant
Depth*Days	0.008	Significant
Treatment*Depth*Days	0.461	NS
Lateral layers	<0.001	Significant
Treatment* Lateral layers	0.630	NS
Days* Lateral layers	0.039	Significant
Treatment*Days* Lateral layers	0.258	NS
Depth* Lateral layers	<0.001	Significant
Treatment*Depth* Lateral layers	0.955	NS
Depth*Days* Lateral layers	0.978	NS
Treatment*Depth*Days* Lateral layers	0.584	NS

In, P-60, there were no significant differences in Kelowna-P between lateral layers at all depths but Kelowna-P decreased with depth for all lateral layers (Fig 3.14, Table 3.21). However, in P-90, there were significant differences in Kelowna-P between the central core and the second torus at all depths. In P-90, Kelowna-P also decreased significantly with depth under all lateral layers (Fig 3.15, Table 3.22). In D-60, there were significant differences in Kelowna-P between the central core and the second torus at all depths except 0-10 cm (Fig 3.16, Table 3.23). In D-60, Kelowna-P also decreased significantly with depth under all lateral layers. In D-90, there were no significant differences in Kelowna-P between lateral layers at all depths except the 20-35 cm depth but Kelowna-P decreased with depth for all lateral layers (Fig 3.17, Table 3.24). The behavior of Kelowna P was quite complex.

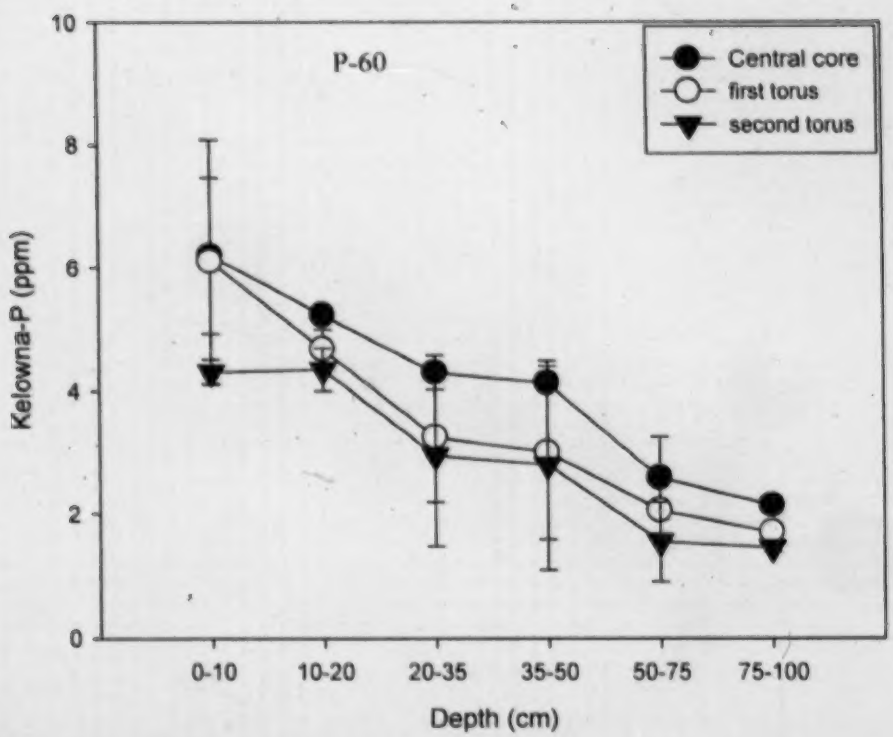

Figure 3.14. Kelowna-P in different depths and lateral layers of soil column at Day 60 with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.21. Kelowna extractable P content at Day 60 in three lateral layers of the six different vertical depths in point application system for 60 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Kelowna-P (ppm)		
	Central core	First torus	Second torus
0-10	6.2 a	6.1 a	4.3 abcd
10-20	5.2 ab	4.6 abc	4.3 abc
20-35	4.3 abcd	3.2 bcdef	2.9 cdef
35-50	4.1 abcde	3.0 cdef	2.8 cdef
50-75	2.5 cdef	2.0 ef	1.5 f
75-100	2.1 def	1.7 f	1.4 f

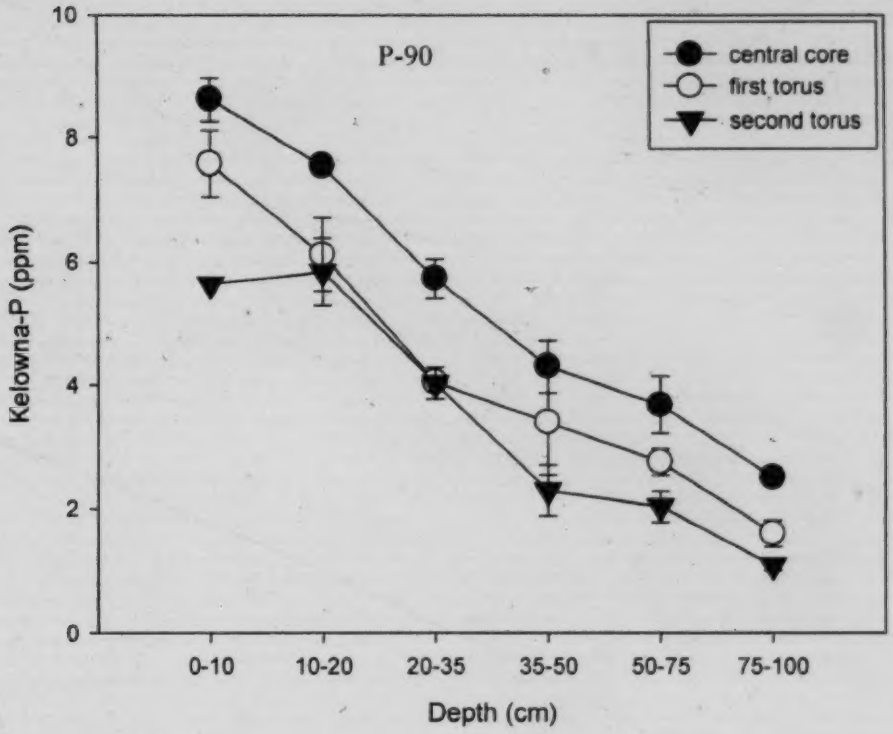

Figure 3.15. Kelowna-P in different depths and lateral layers of soil column at Day 90 with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.22. Kelowna extractable P content in three lateral layers of the six different vertical depths in point application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Kelowna-P (ppm)		
	Central core	First torus	Second torus
0-10	**8.6 a**	**7.5 b**	**5.6 c**
10-20	**7.5 b**	**6.1 c**	**5.8 c**
20-35	**5.7 c**	**4.0 de**	**4.0 de**
35-50	**4.3 d**	**3.4 ef**	**2.2 gh**
50-75	**3.6 de**	**2.7 fg**	**2.0 gh**
75-100	**2.5 g**	**1.6 hi**	**1.0 i**

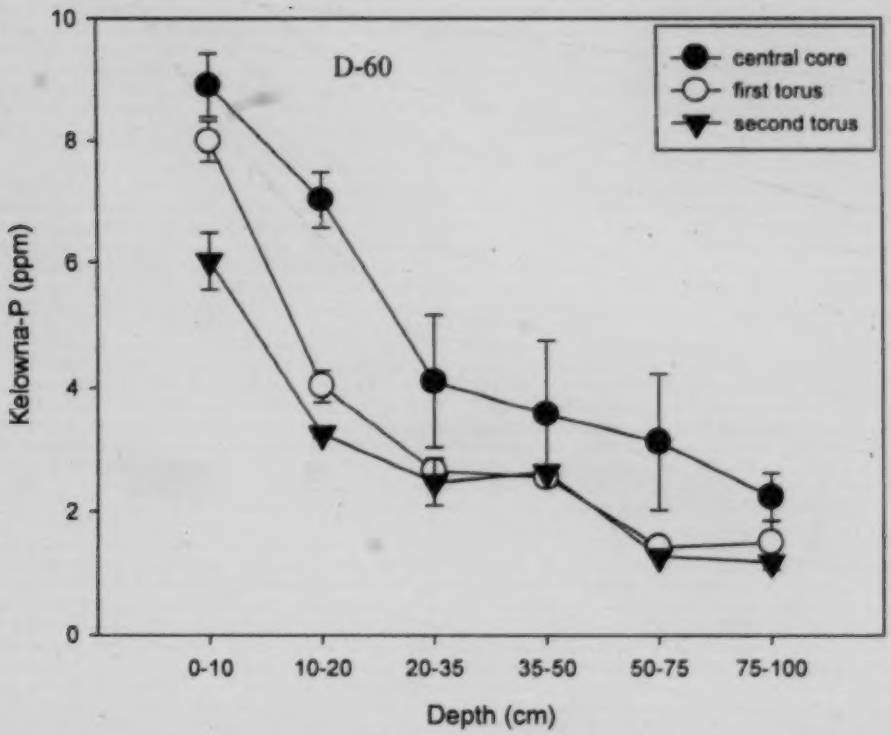

Figure 3.16. Kelowna-P in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.23. Kelowna extractable P content in three lateral layers of the six different vertical depths in drip application system for 60 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Kelowna-P (ppm)		
	Central core	First torus	Second torus
0-10	8.9 a	7.9 ab	6.0 c
10-20	**7.0 bc**	**4.0 d**	**3.2 def**
20-35	**4.1 d**	**2.6 efg**	**2.4 efgh**
35-50	**3.5 d**	**2.5 efg**	**2.6 efg**
50-75	**3.1 def**	**1.4 ghi**	**1.2 hi**
75-100	**2.2 fghi**	**1.5 ghi**	**1.1 i**

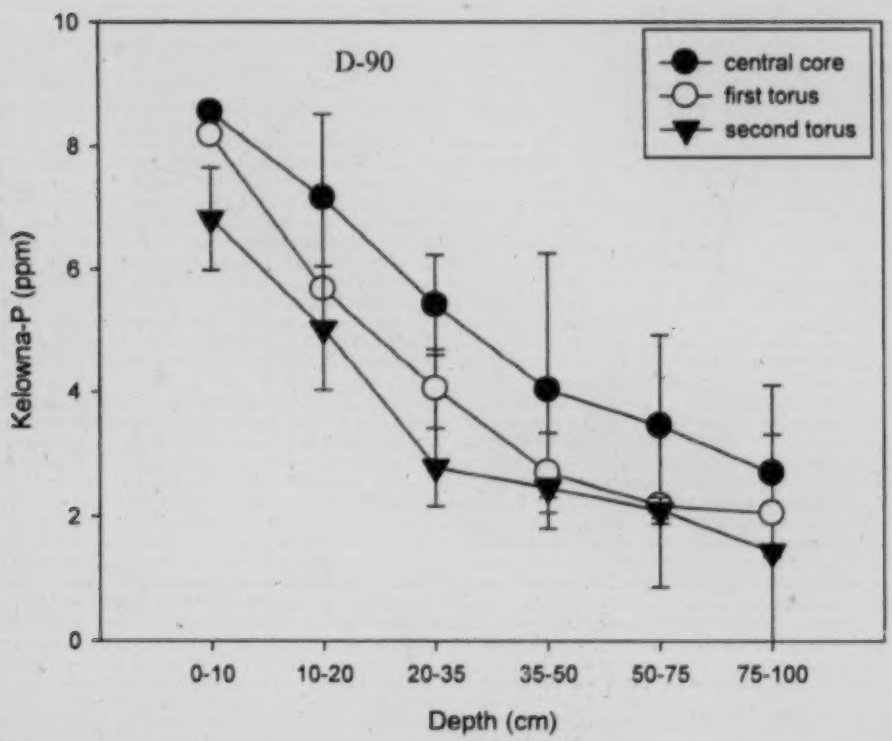

Figure 3.17. Kelowna-P in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.24. Kelowna extractable P content in three lateral layers of the six different vertical depths in drip application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Kelowna-P (ppm)		
	Central core	First torus	Second torus
0-10	8.5 a	8.1 a	6.8 ab
10-20	7.1 ab	5.6 bc	5.0 bcd
20-35	**5.4 bcd**	**4.0 cde**	**2.7 ef**
35-50	4.0 cde	2.7 ef	2.4 ef
50-75	3.4 def	2.1 ef	2.1 ef
75-100	2.6 ef	2.0 ef	1.4 f

3.5.2.5 Fecal coliforms:

Treatments did not have any significant effect on fecal coliforms. Fecal coliforms in soil did not differ significantly for the duration of the experiment and lateral layers. All interactions were also not significant. Fecal coliforms in the soil decreased significantly with depth (Table 3.25).

Table 3.25. Probability of F values from Analysis of Variance of fecal coliforms in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significance at 95% Confidence level
Treatment	0.295	NS
Days	0.568	NS
Treatment* Days	0.663	NS
Depth	0.017	Significant
Depth* Treatment	0.189	NS
Depth*Days	0.768	NS
Treatment*Depth*Days	0.835	NS
Lateral layers	0.070	NS
Treatment* Lateral layers	0.388	NS
Days* Lateral layers	0.263	NS
Treatment*Days* Lateral layers	0.412	NS
Depth* Lateral layers	0.088	NS
Treatment*Depth* Lateral layers	0.243	NS
Depth*Days* Lateral layers	0.386	NS
Treatment*Depth*Days* Lateral layers	0.339	NS

In P-60, the fecal coliforms in the central core at the 10-20 and 35-50 cm depths were significantly higher those in the second torus (Figure 3.18, Table 3.26). Fecal coliforms were not detected in the second torus at a depth of 35-50 cm, and none were detected in the below 50 cm at Day 60. In P-90, the fecal coliforms in the central core at the 1-10, 10-20 and 35-50 cm depths were significantly higher those in the second torus (Figure 3.19, Table 3.27). Fecal coliforms were not detected in the second torus at a depth of 50-75 cm, and none were detected in the below 75 cm at Day 90. Therefore, during the 30 day period between Day 60 and Day 90, fecal coliforms were transported into the lowest layer of the soil column. In D-60, fecal coliforms in the central core were significantly higher those in the second torus at the 0-10 and 10-20 cm depths (Figure 3.21, Table

3.29). Fecal coliforms were not detected in the second torus at a depth of 35-50 cm, and none were detected in the below 50 cm at Day 90.

In D-60, the fecal coliforms in the central core at the 0-10 cm depths was significantly higher those in the first and second torus (Figure 3.20; Table 3.28). Fecal coliforms were not detected in the second torus at a depth of 20-35 cm and none were detected in the below 35 cm at Day 60. In D-90, fecal coliforms were not detected in the second torus at a depth of 35-50 cm and none were detected in the below 50 cm at Day 90.

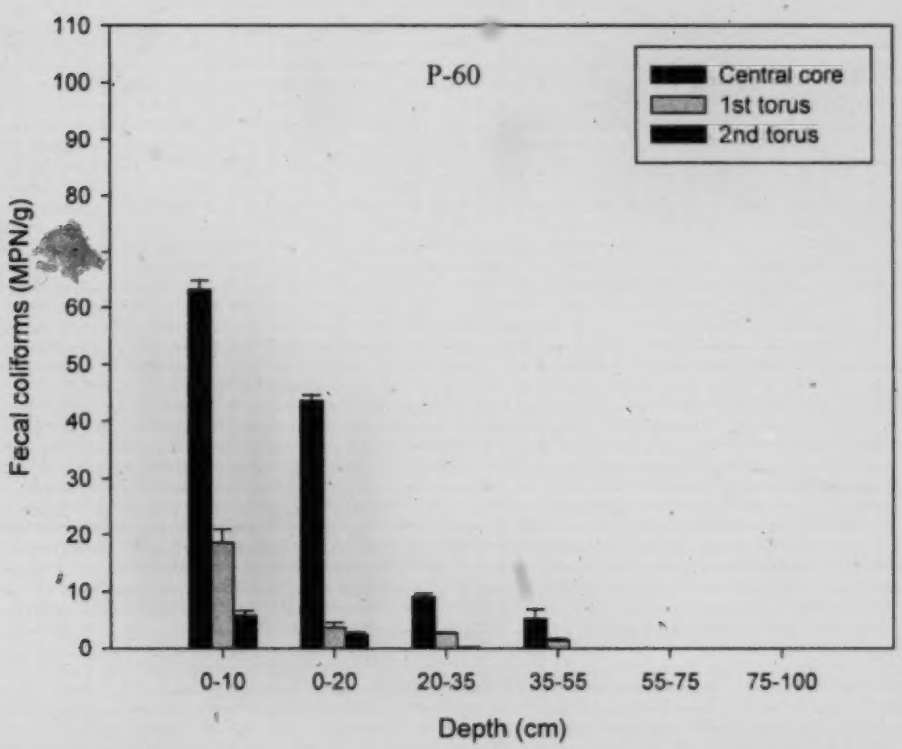

Figure 3.18. Fecal coliforms in different depths and lateral layers of soil column at Day 60 with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.26. Fecal coliforms at Day 60 in three lateral layers of the six different vertical depths in point application system for 60 days. The values are mean of two replicates. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Fecal coliforms (MPN/g)		
	Central core	First torus	Second torus
0-10	63.1 a	18.6 abc	5.8 cde
10-20	**43.7 ab**	**3.6 cde**	**2.3 de**
20-35	9.1 bcd	2.7 de	0.3 f
35-50	**5.1 cde**	**1.4 e**	**0**
50-75	0	0	0
75-100	0	0	0

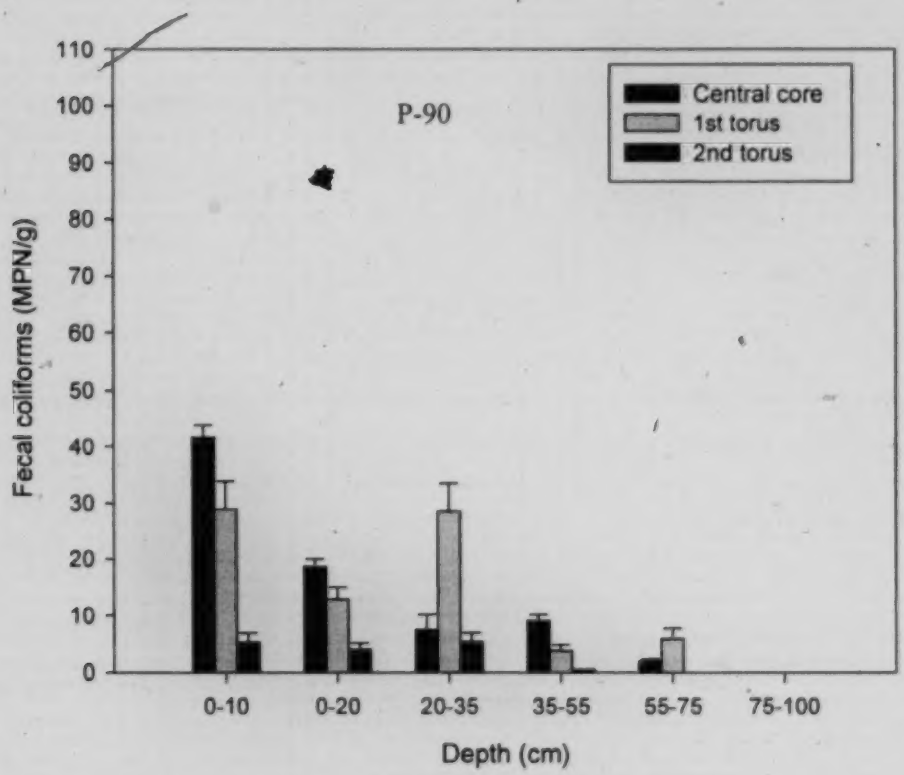

Figure 3.19. Fecal coliforms in different depths and lateral layers of soil column at Day 90 with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.27. Fecal coliforms in three lateral layers of the six different vertical depths in point application system for 90 days. The values are mean of two replicate columns. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Fecal coliforms (MPN/g)		
	Central core	First torus	Second torus
0-10	**41.7 a**	**28.8 ab**	**5.3 bcd**
10-20	**18.6 abc**	**12.9 abc**	**4.0 cd**
20-35	7.4 abcd	28.5 ab	5.4 bcd
35-50	**9.1 abcd**	**3.9 cd**	**0.5 e**
50-75	1.8 de	5.9 bcd	0
75-100	0	0	0

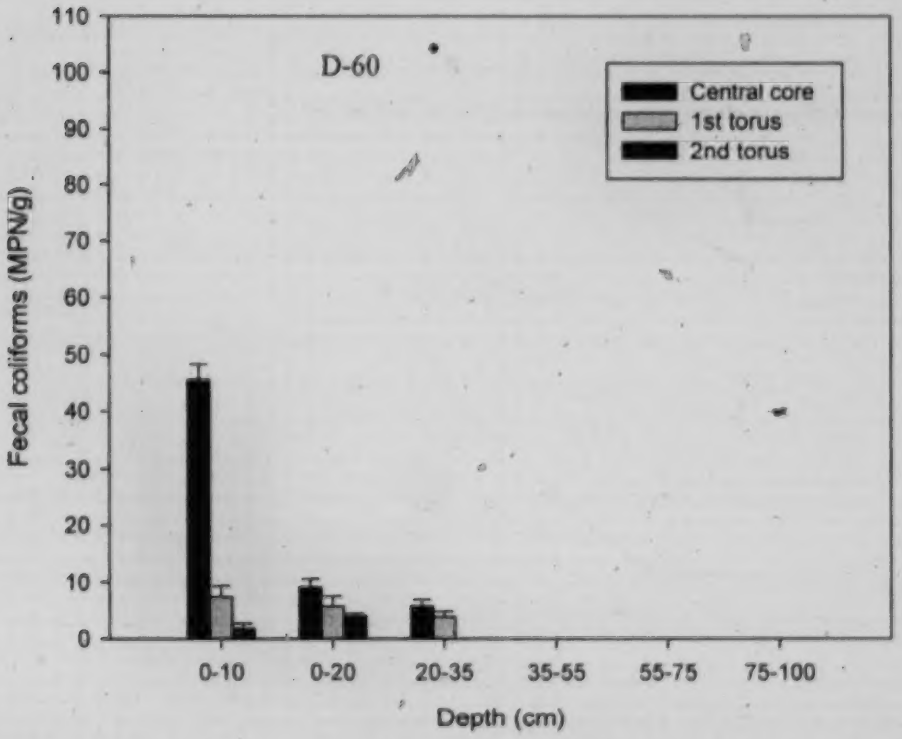

Figure 3.20. Fecal coliforms in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.28. Fecal coliforms in three lateral layers of the six different vertical depths in drip application system for 60 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Fecal coliforms (MPN/g)		
	Central core	First torus	Second torus
0-10	**45.7 a**	**7.4 b**	**1.9 c**
10-20	9.1 b	5.8 b	4.0 b
20-35	5.6 b	3.8 b	0
35-50	0	0	0
50-75	0	0	0
75-100	0	0	0

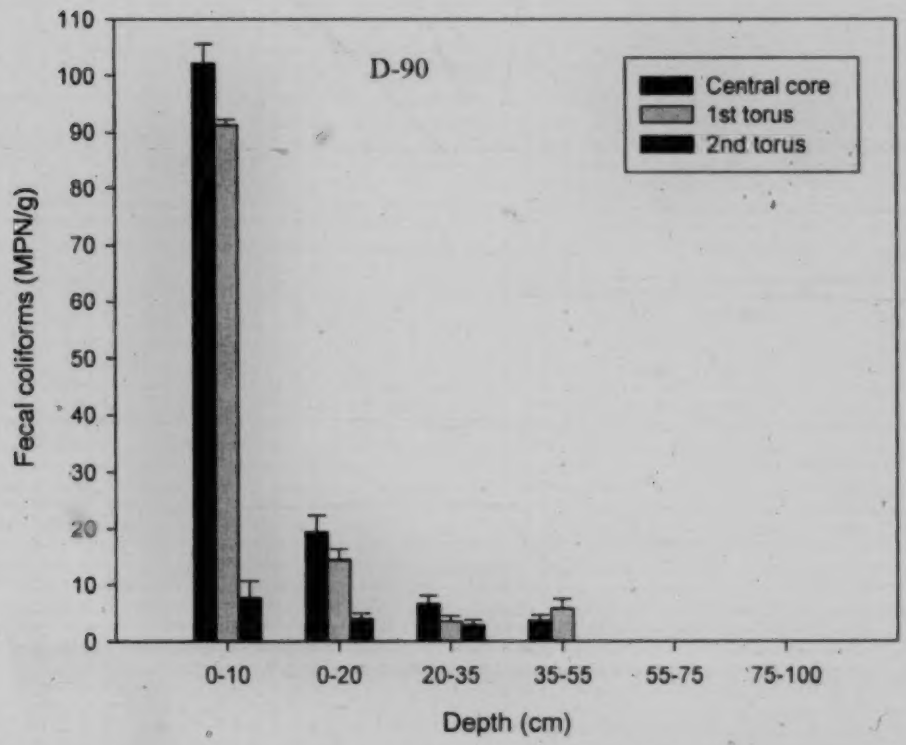

Figure 3.21. Fecal coliforms in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.29. Fecal coliforms in three lateral layers of the six different vertical depths in drip application system for 90 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Fecal coliforms (MPN/g)		
	Central core	First torus	Second torus
0-10	**102.23 a**	**91.20ab**	**7.76 de**
10-20	**19.50 bc**	**14.45 cd**	**3.98 e**
20-35	6.61 de	3.47 e	2.82 f
35-50	3.71 e	5.75 de	0
50-75	0	0	0
75-100	0	0	0

3.5.2.6 Somatic coliphages:

There were many significant effects and interactions for somatic coliphages in the soil columns (Table 3.20). The interactions between treatment and depth, and treatment and lateral layers will be examined through graphs and Duncan's multiple range test comparisons.

Table 3.30. Probability of F values from Analysis of Variance of somatic coliphages in soil columns (Treatment: Point & Drip; Days: 60 & 90 days with 3 lateral layers and 6 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.914	NS
Days	0.009	Significant
Treatment* Days	0.439	NS
Depth	<0.001	Significant
Depth* Treatment	0.644	NS
Depth*Days	0.005	Significant
Treatment*Depth*Days	0.555	NS
Lateral layer	<0.0001	Significant
Treatment* Lateral layer	0.418	NS
Days* Lateral layer	0.0001	Significant
Treatment*Days* Lateral layer	0.304	NS
Depth* Lateral layer	0.006	Significant
Treatment*Depth* Lateral layer	0.496	NS
Depth*Days* Lateral layer	0.079	NS
Treatment*Depth*Days*Lateral layer	0.567	NS

In P-60, there were significant differences in somatic coliphages between the central core and the second torus at all depths except for 10-20 and 75-100 cm (Figure 3.22, Table 3.31). However, differences disappeared and were not significant in P-90 (Figure 3.23, Table 3.32). In all the four treatments, somatic coliphages were present in all the soil layers and at all soil depths with the exception of the second torus of D-60 at a depth of 75-100 cm (Figure 3.24, Table 3.33). In D-90, there were significant differences in somatic coliphages between the central core and the second torus at 0-10 and 10-20 cm depths (Figure 3.25, Table 3.34). Overall, the behavior of somatic coliphages was different from fecal coliforms because of their smaller size.

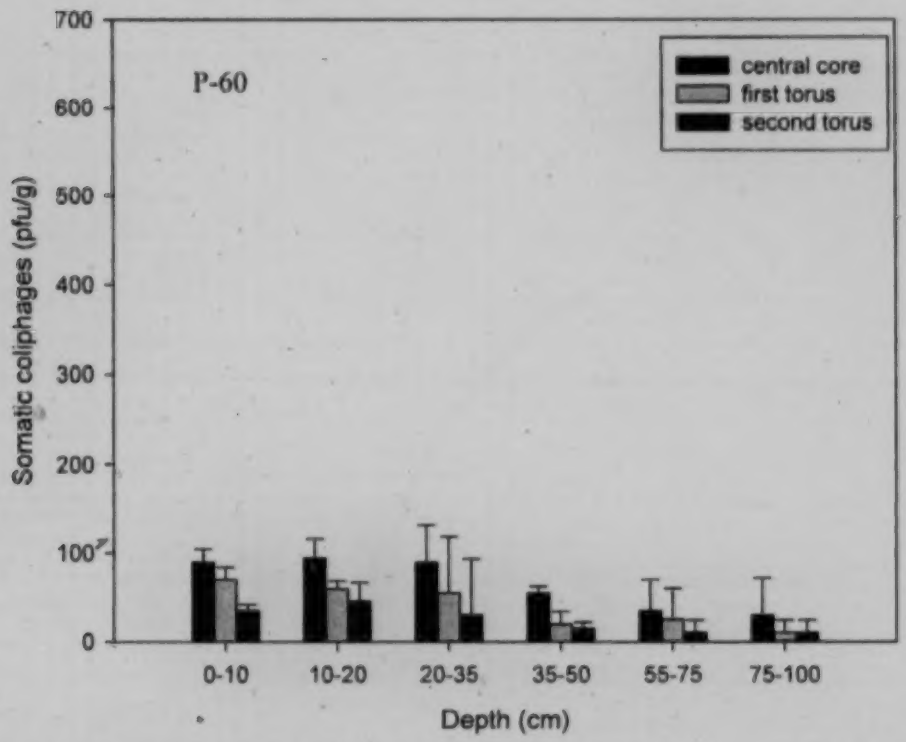

Figure 3.22. Somatic coliphages in different depths and lateral layers of soil column at Day 60 with point application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.31. Somatic coliphages in three lateral layers of the six different vertical depths in point application system for 60 days. The values are mean of two replicate columns. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Somatic coliphages (pfu/g)		
	Central core	First torus	Second torus
0-10	**495.0 a**	**335.0 ab**	**165.00 bc**
10-20	210.0 bc	175.0 bc	100.0 bc
20-35	**325.0 ab**	**200.0 bc**	**75.0 c**
35-50	**150.0 bc**	**100.0 bc**	**60.0 c**
50-75	**125.0 bc**	**95.0 bc**	**50.0 c**
75-100	75.0 c	50.0 c	10.0 c

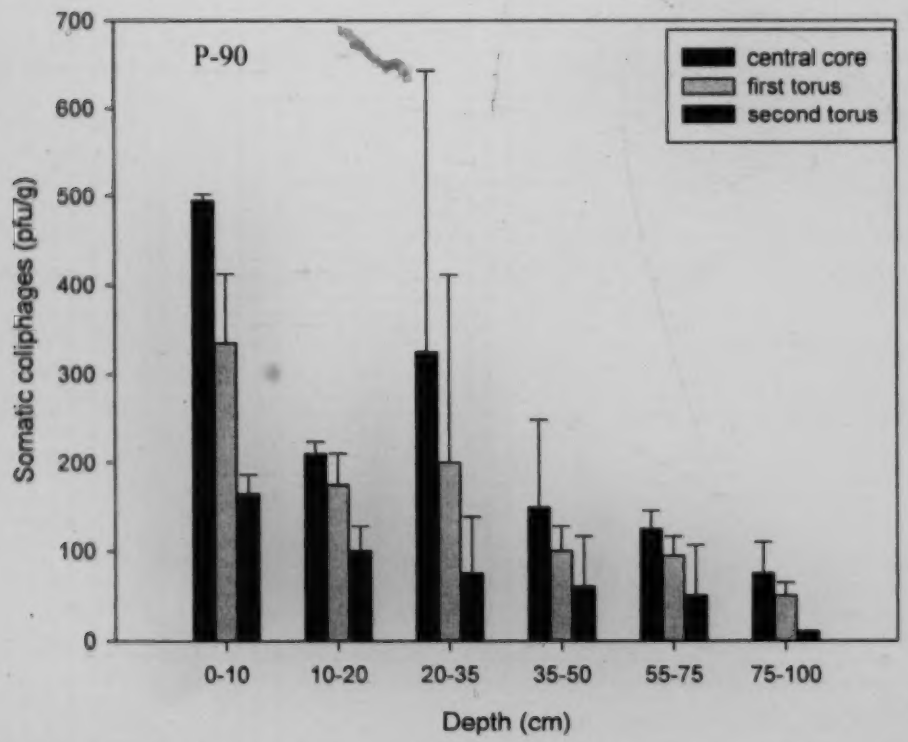

Figure 3.23. Somatic coliphages in different depths and lateral layers of soil column at Day 90 with point application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.32. Somatic coliphages in three lateral layers of the six different vertical depths in point application system for 90 days. The values are mean of two replicate columns. Treatments not followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	Somatic coliphages (pfu/g)		
	Central core	First torus	Second torus
0-10	90.0ab	70.0abc	35.0abc
10-20	95.0a	60.0abc	45.0abc
20-35	90.0ab	55.0abc	30.0abc
35-50	55.0abc	20.0c	15.0c
50-75	35.0abc	25.0bc	10.0c
75-100	30.0abc	10.0c	10.0c

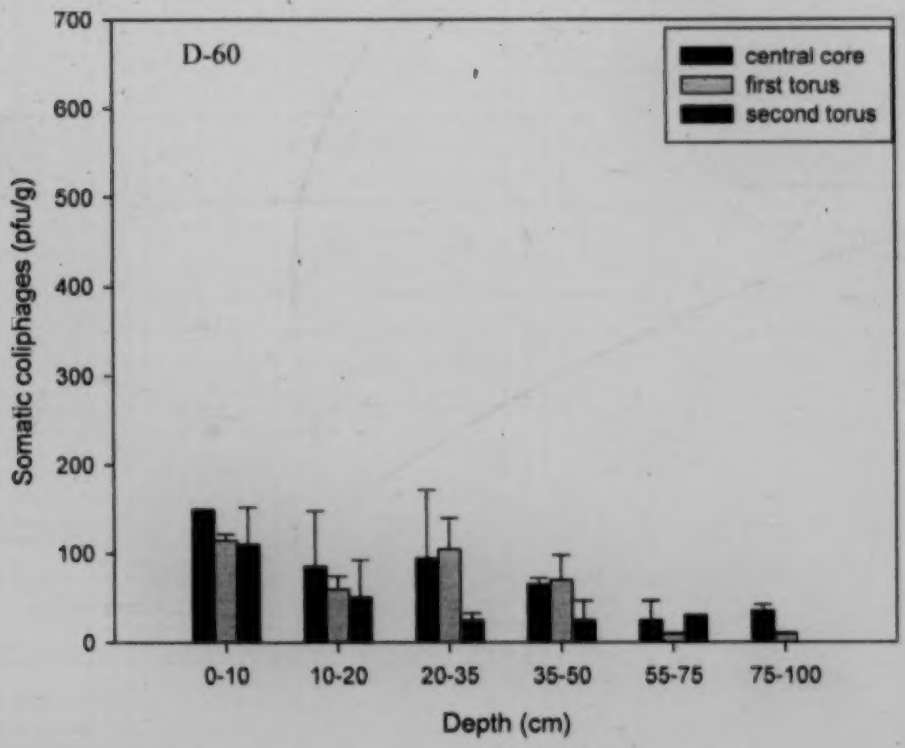

Figure 3.24. Somatic coliphages in different depths and lateral layers of soil column at Day 60 with drip application of effluent for 60 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.33. Somatic coliphages in three lateral layers of the six different vertical depths in drip application system for 60 days. The values are mean of two replicate columns. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Somatic coliphages (pfu/g)		
	Central core	First torus	Second torus
0-10	150.0 a	115.0 ab	110.00 ab
10-20	85.0 abcd	60.0 bcde	50.0 bcde
20-35	95.0 abcd	105.0 abc	25.0 de
35-50	65.0 bcde	70.0 bcde	25.0 de
50-75	25.0 de	10.0 e	30.0 de
75-100	**35.0 cde**	**10.0 e**	**0.0**

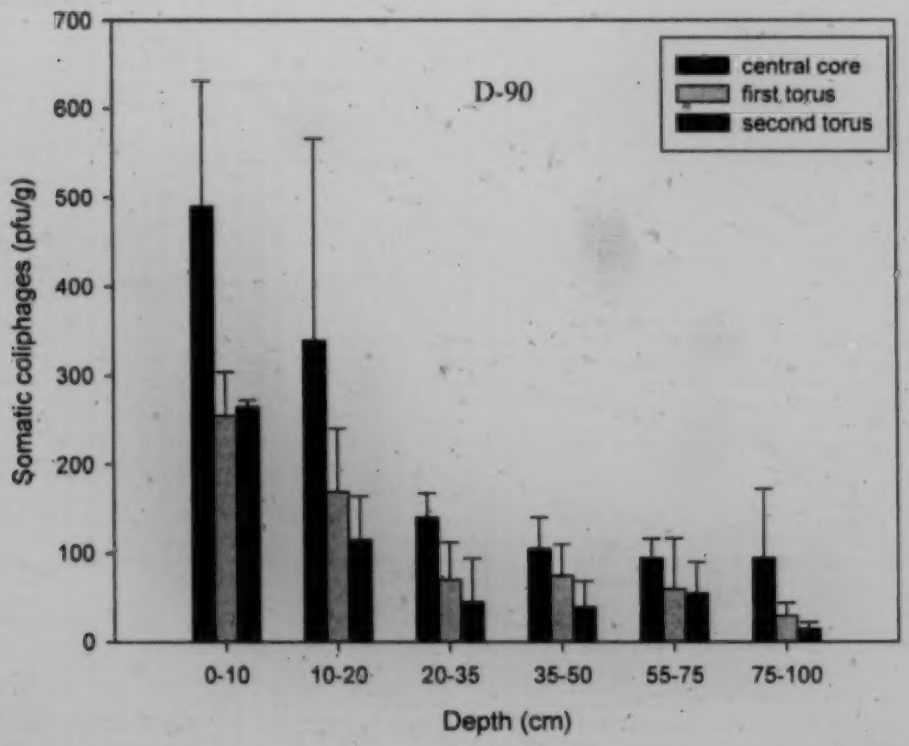

Figure 3.25. Somatic coliphages in different depths and lateral layers of soil column at Day 90 with drip application of effluent for 90 days (mean of 2 columns). Error bar shows standard deviation (±).

Table 3.34. Somatic coliphages in three lateral layers of the six different vertical depths in drip application system for 90 days. The values are mean of two replicate columns with ± standard deviations. Treatments followed not by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Vertical depths (cm)	No. Somatic coliphages (pfu/g)		
	Central core	First torus	Second torus
0-10	**490.0a**	**255.0bc**	**265.0bc**
10-20	**340.0ab**	**170.0cd**	**115.0cd**
20-35	140.0cd	70.0d	45.0d
35-50	105.0cd	75.0d	40.00d
50-75	95.0cd	60.0d	55.0d
75-100	95.0cd	30.0d	15.0d

3.6 Discussion

In this experiment, a point application system was compared to a drip dispersal system to quantify the movement of wastewater, nutrients and indicator microorganisms through soil columns under laboratory conditions.

3.6.1 Comparison of soil moisture regimes in soil columns for the two methods of effluent application

In Figure 2.11, the matric potential and volumetric moisture content data clearly showed that the soil which received DPE by point application was wetter than the soil which received DPE by drip application. For this portion of the study, gravimetric soil moisture content was measured after dismantling the soil columns on Day 60 and Day 90. The soil moisture regime in both treatments ranged from moist to wet. The gravimetric moisture content of the soil did not differ significantly among the treatments but decreased significantly with depth. The moisture content also decreased from central core to second torus in soil columns under drip application system but not for the point application system. Differences can be attributed to the difference in methods of application because in the point application system the dose duration was 1 minute but in the drip application system the dose duration was 5 hours. So, in the point application system, the top soil was saturated for some time. Under these conditions, nutrients and organisms could be transported through all the lateral layers (Toner et al., 1989; Ryan et al., 2001). On the other hand, in drip application system, water could reach through the

lateral layers only through capillary tension. The movement of water in soil is important because water distribution pattern plays an important role for the movement of nutrients (Mahmoud et al., 2007) and organisms through the soil.

3.6.2 Comparison of the movement of ammonium versus nitrate for the two methods of effluent application

Water movement is the major process for the solute and microbial transport through the soil. The initial NH_4-N content of the effluent was 23.7 mg/L (Table 2.2) but in the leachate the content ranged from 0.09 ppm to 0.40 ppm (Table 3.1). The transport of cations, including NH_4-N, are slow in the soil due to sorption to the negative charges of clay particles and soil organic matter while other scientists (Toner et al. 1989; Brooks et al. 1998 and Ryan et al. 2001) reported that leaching of nutrients from wastewater effluent depends on the wastewater flow rate and the strength of sorption to the soil matrix. The NH_4-N content in the upper depths was derived for DPE and decreased with depth. The NH_4-N content decreased significantly ($P=<0.001$) from the central core to the second torus. As the DPE by both application methods was applied into the center of the column NH_4-N was attenuated in the center of the column especially in the upper depths of the soil columns.

On the other hand, NO_3-N in the leachate ranged from 0.20 to 4.68 mg/L (Table 3.2) which was much higher than the initial NO_3-N content of the effluent. Although the soil moisture regime ranged from moist to wet, it is quite possible that the soil got aerated during the periods when the DPE was not being applied. Nitrification causes the conversion of NH_4-N to NO_3-N, an anion and is not adsorbed by the soil particles.

Brown et al (1977) and Reneau et al (1985) reported that under reduced (anaerobic) conditions, NH_4-N accumulated in soils and moved only about as far and as fast as phosphates but when the soil was allowed to become oxidized large amounts of NH_4-N were converted to NO_3-N which rapidly leached to the groundwater.

The present study support the findings of Reneau et al. (1985) and Brown et al. (1977). In this experiment, the effluent dose was once per day in both application systems that could allow soil to have unsaturated flow and also the water content throughout the experiment was near or below the saturation point (Figure 2.9). That allows to NH_4-N to oxidized to NO_3-N.

The higher amount of NO_3-N obtained in the leachate might be due to the conversion NH_4-N to NO_3-N in soil column. Iskander et al. (1980) found in their experiment working with effluent that NH_4-N content was much higher than the NO_3-N content of the column soil. There is a possibility that some of NO_3-N could also have been denitrified. Livesley et al. (2007) reported that the downward movement of N under effluent application was dominated by NH_4-N rather than NO_3-N.

3.6.3 Comparison of the movement of Kelowna-P for the two methods of effluent application

Phosphate, the other common contaminant of domestic wastewater, is readily absorbed in the soil. The water-soluble phosphate in the leachate ranged from 0.07-0.21 mg/L (Table 3.3) that was much lower than the initial PO_4 content of the effluent. This might be due to that PO_4 was adsorbed by the soil. In the soil, the Kelowna-P content decreased with increasing vertical depths and from central core to second torus. In the soil, higher concentration of Kelowna-P is accumulated in the upper vertical depths than the lower depths and central core to second torus due to its insolubility in water (Gerritse, 1993; 1994) also found the similar results in his experiments.

3.6.4 Comparison of the movement of fecal coliforms and somatic coliphages for the two methods of effluent application

Fecal coliforms were not found in the leachate during the course of this experiment. It is well known that the soil has an adsorption capacity for microorganisms such as fecal coliforms (Tim et al., 1988). In the present experiment, fecal coliforms were detected up to 60 cm of depth after dosing the soil continually for 90 days.

Although the transport of fecal bacteria is affected by the factors such as cell size, cell surface properties, soil type, pH of the solution, ionic strength, mobility of the bacterium, grain size, permeability, porousness and clay mineralogy (Sharma and Mc Inerney 1994: Gannon et al., 1991a, 1991b; Harvey et al., 1993; Jewett et al., 1995 and Mc Caulou et al., 1995), fecal coliforms moved from the surface to the subsurface. They could have moved with water which took them into deeper depths (Hendry et al., 1999).

Somatic coliphages were detected in the leachate during the experiment. Kouznetsov et al., (2004) reported that somatic coliphages are the most persistent and

mobile microorganisms due to their lower adsorption and die-off. It has also been reported that high water content affects the adsorption and desorption of somatic coliphages in soil (Lance et al., 1976) and they can travel long distance with high flow rate of water (Lance and Gebra, 1984). In another experiment, Gantzer et al., (2001) found that somatic coliphages travel longer distance and survive much longer time than the fecal coliforms. In the soil, the somatic coliphages number decreased significantly with increasing vertical depths and from central core to second torus. This would happen due to the amount and flow rate of water in the column (Malcolm et al., 2001). So, the acceptable performance of a soil system has to be stop migrating the bacteria and viruses within one meter depth.

3.7 Conclusions

The following conclusions are drawn from the experiment:

- NH_4-N, NO_3-N, water soluble-P and somatic coliphages were detected in leachate samples throughout the experimental period but fecal coliforms were not detected in the leachates.
- NH_4-N, NO_3-N and Kelowna-P in soil decreased significantly ($P=<0.05$) with depth and from central core to second torus in both application methods.
- During the 60 to 90 day period, there was movement of fecal coliforms in soil columns from 35-55 cm to 55-75 cm depth in the point method and from 20-35 to 35-55 cm depth in the drip method.
- Fecal coliforms and somatic coliphages decreased significantly ($P=<0.05$) from central core to second torus in both application systems.

3.8 Design Implications

The onsite industry has to develop better designs for dispersing wastewater in soils. Some important factors that should be considered are: loading rates based on soil type, orifice spacing, application methods, size and shape of chambers, and direction of orifices on the laterals. This project has partially addressed the issues of application methods at one loading rate based on soil type. Further research is needed to develop new designs.

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Chapter 4

Movement of water, nutrients, fecal coliforms and somatic coliphages in a field soil receiving wastewater through point and drip application methods

4.1 Introduction:

The laboratory soil column experiment presented in Chapters 2 and 3 was designed to mimic the dynamics of effluent being applied from an orifice in a lateral of an at-grade, soil-based dispersal system and that of effluent being applied through a dripper from a drip line. This experiment has provided insights into the lateral and vertical flow of wastewater, nutrients, fecal coliforms and somatic coliphages through different soil layers which corresponded to major soil horizons of a Dark Gray Luvisol. In order to extend the laboratory study to a field scale, the experiment was conducted at the Ellerslie Experimental Research Station. An additional tracer in form of a dye was used to track the movement of diluted primary effluent (DPE). The appropriate literature review for this tracer technique is presented below.

4.2 Literature Review

4.2.1 Tracking preferential flow under field conditions:

Preferential flow describes the physical phenomenon of rapid transport of water and solutes in soil that occurs in most soils (Flury and Flühler, 1994). Preferential flow or macropores flow, also known as short-circuiting, refers to rapid, downward movement of water through large, vertical pores, bypassing slowly permeable, adsorptive soil peds. Macropore flow involves transport through non-capillary cracks or channels within a profile, reflecting soil structure, root decay, or the presence of wormholes, and of ant or termite tunnels. One important characteristics of preferential flow is that solutes by pass a large part of soil matrix. Thus, strongly sorbing compounds like pesticides may be more mobile than anticipated and provide a possible hazard for ground and surface waters (Flury, 1996). Additionally, sorption and degradation rates of preferential flow paths and soil matrix may be different due to different microbial populations (Pivetz and Steenhuis,

1995). Therefore distinct differences in physicochemical properties between preferential flow paths and soil matrix can be expected.

4.2.2 Use of Brilliant Blue FCF Dye

Brilliant Blue FCF has been used in several field experiments due to its good visibility, low toxicity, and weak adsorption on soils (Flury and Flühler, 1994; Aeby et al. 2001). Recent investigation showed that the adsorption of the dye differs between soil types (Ketelsen and Meyer-Windle, 1999). Soils with high clay and low organic content tend to absorb more dye than others. A recommended range for good strain visibility is 3-5 kg/m^3 (Flury and Flühler, 1994).

4.2.3 Innovative Use of Brilliant Blue FCF Dye for Wastewater Research

Previous research on at-grade dispersal fields has shown that the dominant factor for wastewater flow under the orifice of a lateral is downwards (Juma et al., 2007). In order to demonstrate this under field condition, the dye could be added to DPE and added to the research microplots on a continual basis. If enough dye is added over a short of period of time, the pattern of wastewater flow in soil could be visually identified. If one assumes that fecal coliforms and somatic coliphages also move with the wastewater, then it should be possible to quantify their numbers in stained and unstained soil. Therefore, there is a great potential to use this technique to quantify the lateral and vertical transport of wastewater, nutrients and microorganisms added to soil by point and drip application methods under field condition.

4.3 Objective

The objective of this research was to quantify the lateral and vertical distribution and transport of wastewater, nutrients (NH_4-N, NO_3-N and Kelowna extractable P), fecal coliforms and somatic coliphages in an Eluviated Black Chernozem dosed with dye-enhanced wastewater applied by point and drip application methods.

4.4 Materials and Methods:

4.4.1 Experimental Site:

The experiment was conducted at Ellerslie Research Station, which is located 15 km SW of the University of Alberta campus in Edmonton, Alberta. The dominant soil on this is an Eluviated Black Chernozem. The physico-chemical properties of this soil are presented in Table 4.1.

Table 4.1. Physical and chemical properties of the Eluviated Black Chernozem at Ellerslie Research Station, Alberta.

Depth cm	pH	EC dS/m	Sand	Silt	Clay	Class	TC	TN
			%				%	
0-10	5.6	0.051	26	56	18	SiL	4.50	0.42
10-20	5.6	0.048	21	61	18	SiL	4.41	0.40
20-30	5.4	0.046	21	52	27	SiL	3.75	0.38
30-40	6.4	0.042	21	51	28	SiL	2.50	0.26
40-60	6.8	0.040	17	53	30	CL	1.50	0.16
60-80	6.9	0.039	17	53	30	CL	1.45	0.13
80-100	7.1	0.039	16	54	30	CL	1.40	0.12

4.4.2 Dye Tracer:

The dye tracer used to identify the flow paths of effluent was Brilliant Blue FCF (C. I. 42090, Erioglucine; Acid Blue 9, $C_{37}H_{34}N_2O_9S_3Na_2$, FW 792.86). The dye was chosen due to its low toxicity and good visibility in soil materials and because it absorb weekly on soils (Flury and Fluhler, 1994; Neurath el al., 2005). The Brilliant Blue FCF is a food grade chemical. The concentration of dye used was 4 kg/m^3 in the effluent.

4.4.5. Effluent Collection and Concentrations:

(a) The Primary effluent was collected from the Gold Bar Wastewater Treatment Plant in Edmonton twice a week and was stored at 4°C. It was diluted 3-fold to simulate household effluent. As the soil at Ellerslie has high biological activity, a higher strength of effluent was used but the TSS and BOD_5 values were still in the range of secondary treated effluent.

The Chemical and microbiological properties of the primary and diluted effluent are given in Table 4.2.

Table 4.2. Chemical and microbiological properties of primary and diluted effluent.

Type	pH	EC dS/m	TSS mg/L	BOD_5 mg/L	Fecal Coliforms Log MPN/100 ml	Somatic Coliphages pfu/100 ml	NH_4-N mg/L	NO_3-N mg/L	PO_4 mg/L
Primary	7.09	1.20	80	130	7.36	1500	108.65	0.60	20.01
Diluted (1:3)	6.89	0.51	28	40	5.79	855	23.73	0.18	6.06

4.4.6 Field experiment:

In order to determine the water distribution pattern, movement of nutrients, fecal coliforms and somatic coliphages an experiment was setup in the field condition in the University of Alberta Experimental Farm (Ellerslie, Edmonton) where the effluent was applied by point and drip application methods. The experiment consists of point and drip application of diluted primary effluent (DPE) with 2 replications.

4.4.7 Effluent application:

The dilute primary effluent applied by point and drip applications system. The vegetations on the field site were removed manually to minimize the disturbance and ensure the uniform applications of effluent. Four rectangular metal frames (42 cm x 42 cm x 10 cm height) (Figure 4.1) were inserted in the soil to a depth of 5 cm. Thus, the metal frame acted as an open ended container to reduce the lateral loss of the effluent.

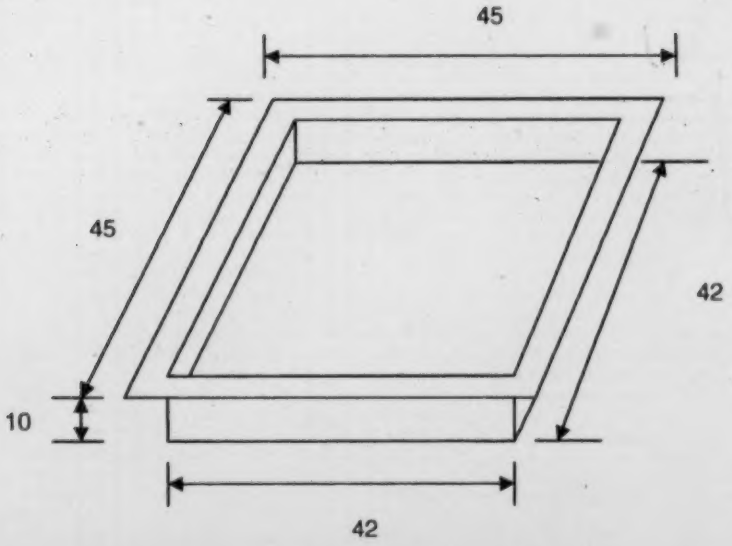

Figure 4.1. Metal frame used in the field dye experiment.

Photo 4.1. Experiment site covered with polyethylene sheets, and plastic container and funnel used apply the DPE by point application method.

Photo 4.2. Plastic containers for drip application of the DPE were supported above the soil surface with a wooden frame and planks (left); the experiment site after adding Brilliant Blue dye (right). The two microsites in the foreground received DPE by the drip method while the two in the background received DPE by the point method.

Diluted primary effluent (DPE) was stained with Brilliant Blue dye to yield an effluent with a concentration of 4 kg/m^3. The duration of the experiment was 15 days. In case of the simulation of point application, the DPE was added through a funnel, which was located 5 cm above the surface. The dose volume was 1500 ml/day. The flow rate of the DPE application was 1500 ml/minute for point application and 1.66 ml/min for drip application, respectively. To simulate drip application, the DPE was put into plastic containers, which had pipette tips attached to the bottom. The containers were held in place by strings knotted at the handles of the container. The containers were supported by planks of wood so that the pipette tips were 5 cm above the soil surface. The tips were calibrated to deliver DPE at the desired rate.

4.4.8 Soil Sampling Preparation:

Soil microplots were sampled after a day of the final dye application. A soil pit was dug at one edge of the metal frames. The pit was shaved to the edge of the metal frame and then the metal frames were removed. A photo of the site is presented in Photo 4.2

4.4.9 Soil Sampling Scheme:

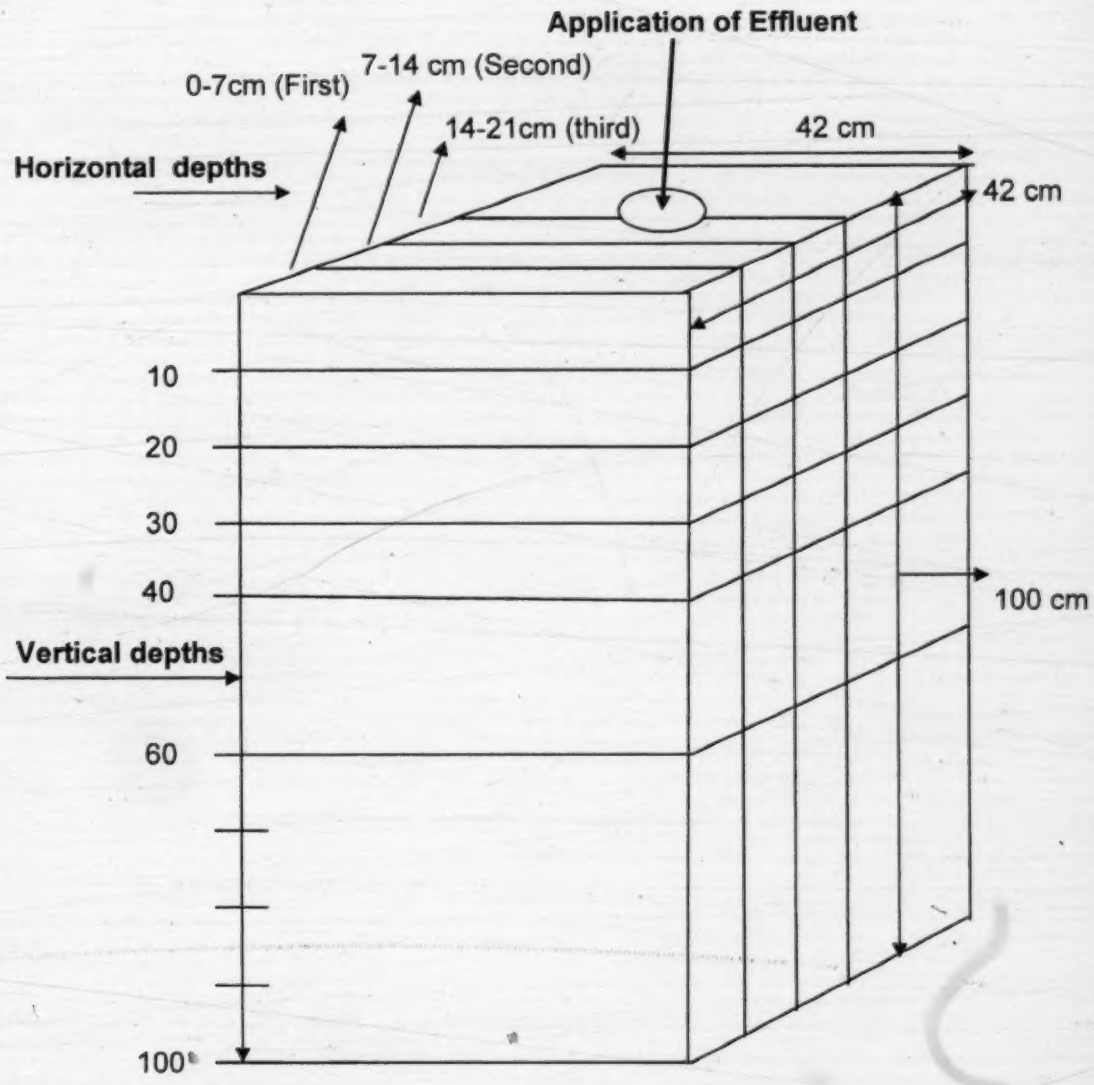

Figure 4.2. A schematic diagram of the field microsite showing the sampling scheme relative to the point of application of DPE.

The sampling scheme is shown in the Figure 4.2. The dyed and the un-dyed soil blocks from the 3 horizontal layers (0-7, 7-14, and 14-21 cm lateral depths) and 6 depths (0-10, 10-20, 20-30, 30-40 and 40-60 cm) were sampled. The soil sampling procedure was to take samples from the first lateral layer (0-7 cm) from the face of the pit in 10 cm depths and progress vertically downward to a depth of 60 cm because this depth was deeper than the penetration of the dye. The dyed and un-dyed soil samples from each grid layer were taken using sterile technique. The process was repeated for the other two horizontal layers. Photographs were taken of each soil profile to visualize the area of effluent distribution of the point and drip application system. The dyed and un-dyed soil samples were packed in two separate Ziploc bag to prevent moisture loss. Just after sampling, the soil samples were kept in a cooler at 4°C for microbiological analysis. Some parts of the soil samples were air dried and passed through 2 mm sieved for chemical analysis.

4.4.10 Chemical Analysis:

The pH of the soil samples was determined in 1:2.5 soil: water suspension and electrical conductivity (EC) was determined in the supernatant liquid of 1:1 soil: water using an Accumet Research AR20 pH/conductivity meter (Fisher Scientific, Model No. S/N AR 93316577) to characterize the soil. NO_3-N and NH_4-N of the soil samples were extracted with 2M KCl and then analyzed by Ion chromatography (Westco SmartChem 200, Westco Scientific, USA).

4.4.11 Microbiological Analysis:

Somatic coliphages was quantified by the standard procedure (APHA, 2005) using *E. coli* CN (ATCC 600709) strain. The single layer agar technique was used for this determination. Fecal coliforms was also determined by the standard methods described in APHA (2005). Dilutions of the soil were conducted by Phosphate Buffer Solution (PBS). The procedures are described in a greater detail in Chapter 3.

4.4.12 Experimental design:

The experimental design is a complex split plot design. It has 3 sizes of experimental units. The field soil is the experimental units to which treatment (point and drip application of effluent) and dye (stained and non-stained portion) are applied, which are arranged in a completely randomized design structure. The section of a field soil (different depths) is the experimental unit for depth, and the section of a depth is the experimental unit for lateral depths. So, the experiment had two treatments, 2 replications, 3 lateral layers, 6 depths, stained and non-stained soil.

4.5 Results:

4.5.1 Water distribution pattern:

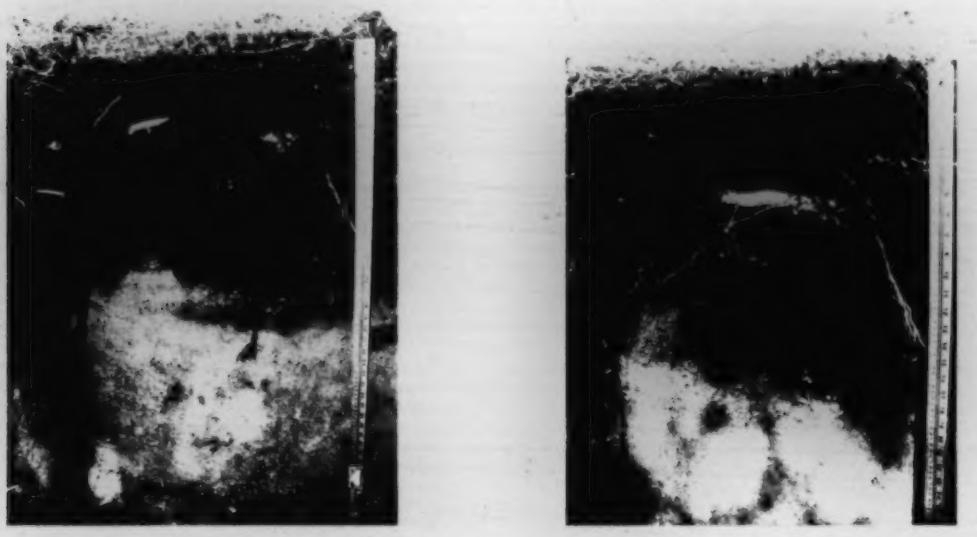

Photo 4.3. Water distribution in the microsites receiving DPE by the point method.

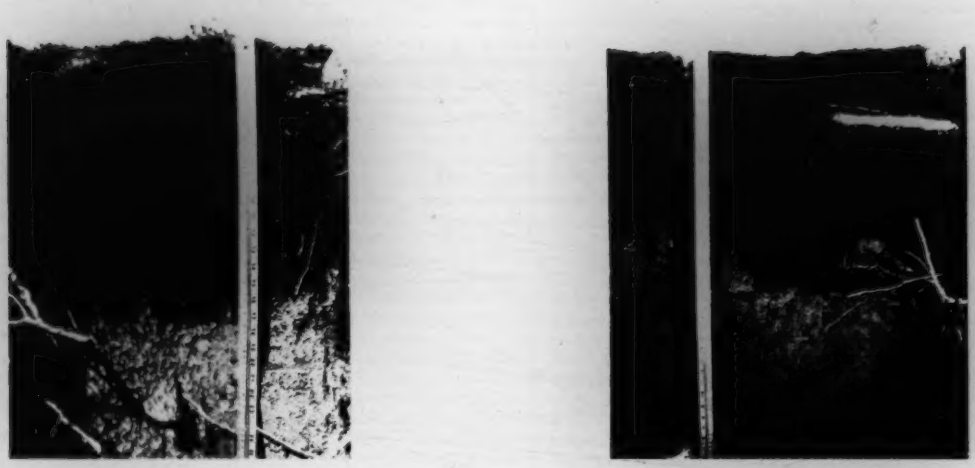

Photo 4.4. Water distribution in the microsites receiving DPE by the drip method.

Water distribution pattern in the soil profile for point and drip application of wastewater can be explained from the above photographs. The dye was added with the effluent to predict the water movement. It was found that in drip application systems the water distribution was more even than point application system. In both application systems the vertical flow of water was higher than lateral flow. On average, the infiltrated stained wastewater was transported vertically to an average depth of 49 cm laterally to 31.5 cm in the point application. In contrast, the infiltrated stained wastewater was transported vertically to an average depth of 44 cm laterally to 28.5 cm in the drip application. Therefore, in the point application method, the wastewater spread more from the point of application and went to a deeper depth. There was uneven distribution of the effluent in the point application method compared to the drip application method.

4.4.2 Gravimetric water content:

ANOVA showed that soil depth, depth*treatment and depth*stain had a significant effect on the gravimetric moisture content (Table 4.3).

Table 4.3. Probability of F values from analysis of variance of gravimetric water content in the field experiment (Treatment: Point and Drip; Stain: stained and non-stained soil; 3 lateral soil layer; 5 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.718	NS
Stain	0.821	NS
Treatment* Stain	0.871	NS
Depth	<0.001	Significant
Depth*Treatment	<0.001	Significant
Depth* Stain	<0.001	Significant
Treatment* Stain *Depth	0.134	NS
Lateral layer	0.133	NS
Treatment*Lateral layer	0.063	NS
Stain *Lateral layer	0.554	NS
Treatment* Stain *Lateral layer	0.178	NS
Depth*Lateral layer	0.528	NS
Treatment*Depth*Lateral layer	0.456	NS
Depth* Stain *Lateral layer	0.560	NS
Treatment*Depth*Lateral layer	0.124	NS

In general, the amounts or concentrations of major variables decreased with depth. A depth*treatment interaction shows that the variable was affected by the treatment when the depth was considered. The stained volume of the soil delineates the extent of capillary suction which effectively equalized the value of the variable. These values were significantly higher than the unstained soil volume.

In the P-stained soil, there were no significant differences between the lateral layers but the moisture content decreased with depth. This means that capillary suction extended over a radius of about 21 cm from the point of application (Figure 4.3; Table 4.4). In the P-non stained soil, which was collected from the identical layers of soil, there

were no significant differences in moisture content between layers. Effectively, these soil samples were outside the influence of the applied effluent (Figure 4.4; Table 4.5).

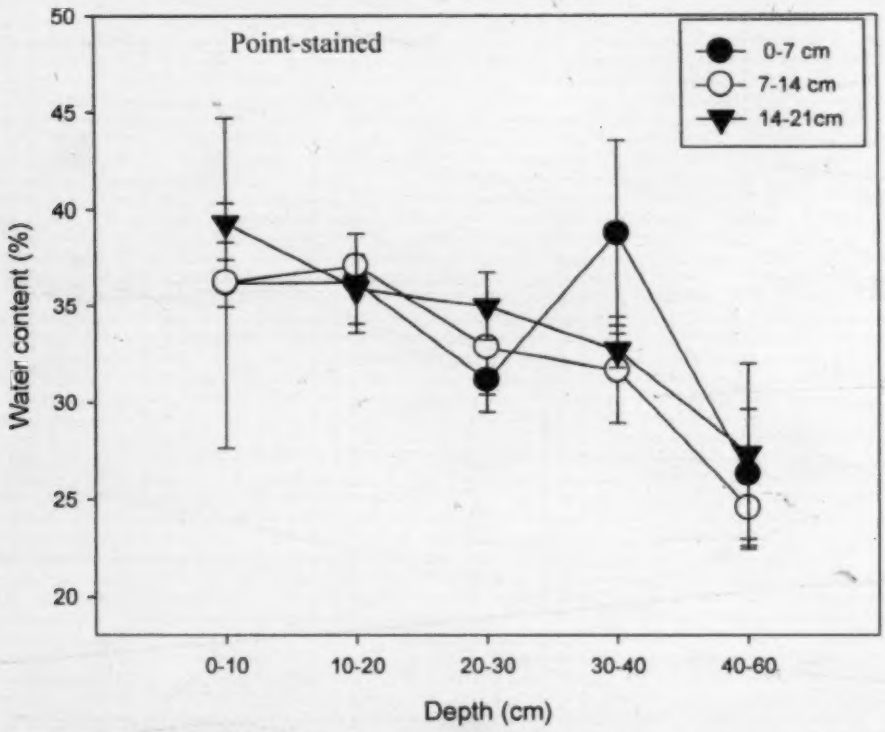

Figure 4.3. Average gravimetric water content in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.4. Average gravimetric water content in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Gravimetric water content (%)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	36.2 ab	36.2 ab	39.3 a
10-20	36.2 ab	37.1 ab	35.9 ab
20-30	31.2 bcde	32.9 abcd	35.0 abc
30-40	38.8 ab	31.7 abcde	32.7 abcd
40-60	26.3 de	24.6 e	27.3 cde

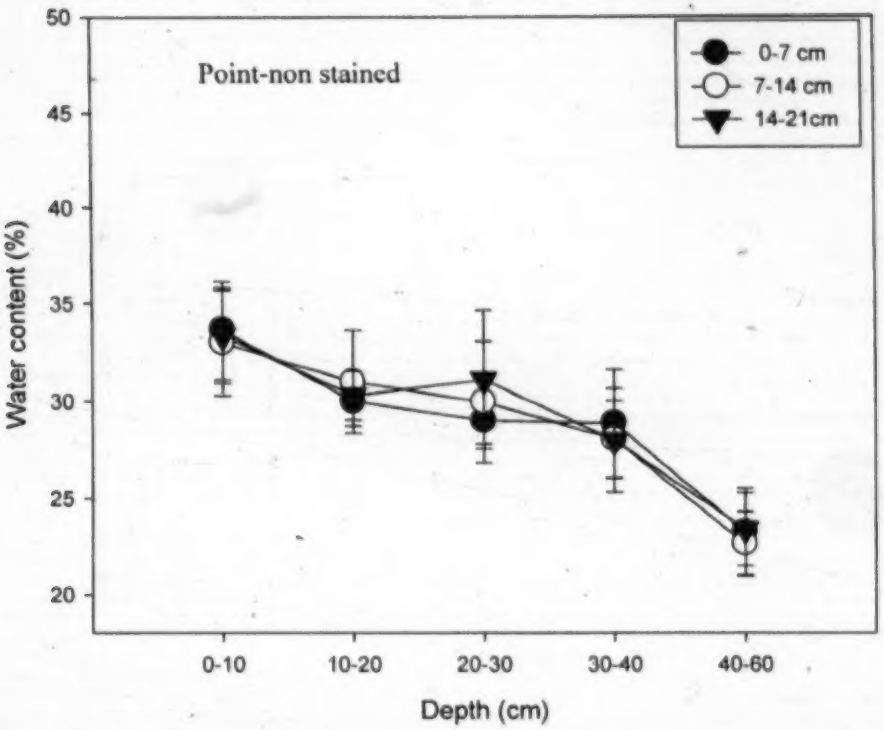

Figure 4.4. Average gravimetric water content in five different depths and three different lateral layers of the non stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.5. Average gravimetric water content in five different depths and three different lateral layers of the non stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Gravimetric water content (%)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	33.6 a	33.0 a	33.4 a
10-20	30.0 bcd	31.0 ab	30.3 bcd
20-30	29.0 bcd	30.0 bcd	31.1 abcd
30-40	28.9 bcd	28.1 cd	28.0 cd
40-60	23.3 e	22.7 e	23.4 e

In the D-stained soil, there were no significant differences between the lateral layers but the moisture content was significantly higher in the 10-20 cm depth compared to 0-10 cm and then it decreased with depth. This means that capillary suction extended over a radius of about 21 cm from the point of drip application (Figure 4.5; Table 4.6). In the D-non stained soil, there were no significant difference in moisture conent between layers and moisture content decreased with depth (Figure 4.6; Table 4.7).

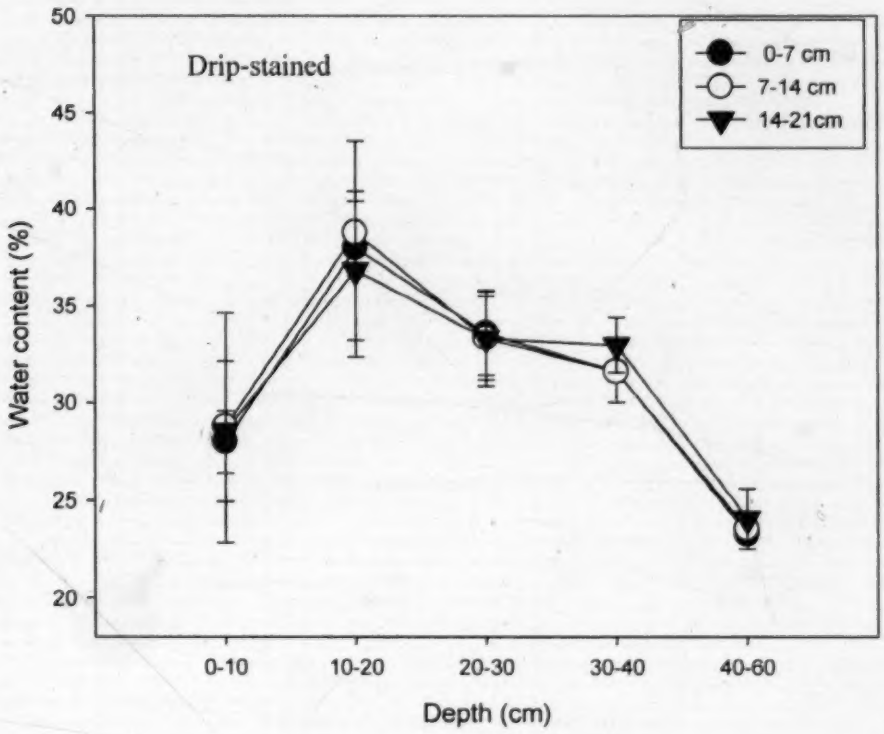

Figure 4.5. Average gravimetric water content in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.6. Average gravimetric water content in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Gravimetric water content (%)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	28.0 f	28.8 e	28.6 ef
10-20	**38.0 ab**	**38.7 a**	**36.8 b**
20-30	33.6 c	33.3 cd	33.3 cd
30-40	31.6 d	31.6 d	33.0 cd
40-60	23.3 g	23.6 g	24.1g

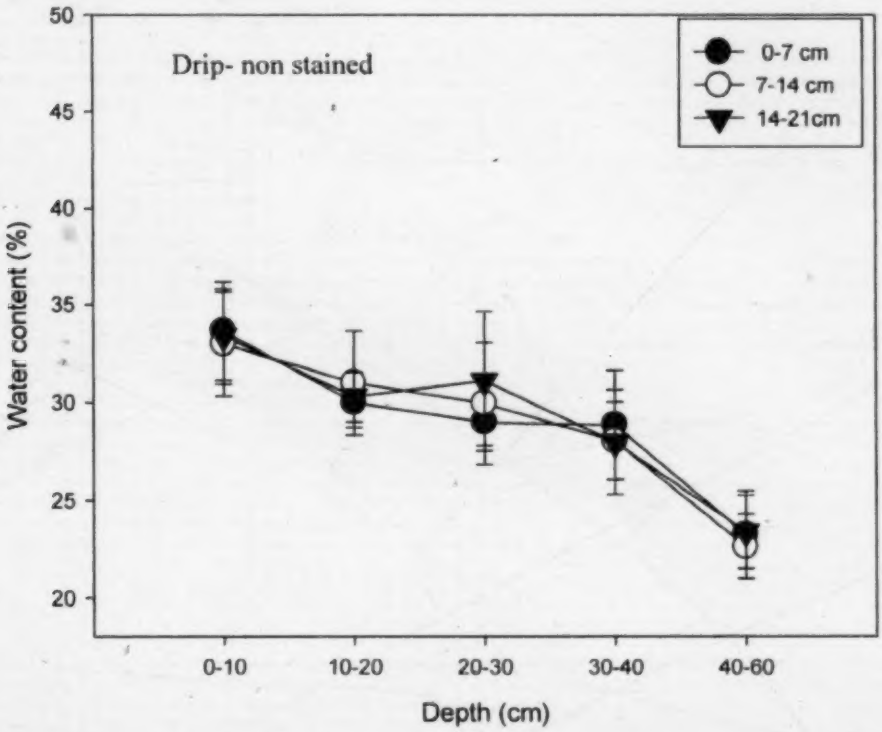

Figure 4.6. Average gravimetric water content in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.7. Average gravimetric water content in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Gravimetric water content (%)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	34.0 a	33.2 ab	33.6 ab
10-20	31.2 abc	32 abc	31.1 abc
20-30	31.2 abc	31.1 abc	31.7 abc
30-40	30.0 bc	30.9 bc	28.4 bc
40-60	26.1 cd	22.2 cd	25.9 cd

4.4.3 NH_4-N content:

The NH_4 content of the soil ANOVA revealed that the depth, stain and depth*stain interaction was significant (Table 4.8)

Table 4.8. Probability of F values from analysis of variance of NH_4-N in the field experiment (Treatment: Point and Drip; Stain: stained and non stained soil; 3 lateral soil layer; 5 vertical depths

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.403	NS
Stain	0.001	Significant
Treatment* Stain	0.822	NS
Depth	<0.001	Significant
Depth*Treatment	0.172	NS
Depth* Stain	<0.001	Significant
Treatment* Stain *Depth	0.356	NS
Lateral layer	0.133	NS
Treatment*Lateral layer	0.068	NS
Stain *Lateral layer	0.936	NS
Treatment* Stain *Lateral layer	0.975	NS
Depth*Lateral layer	0.581	NS
Treatment*Depth*Lateral layer	0.807	NS
Depth* Stain *Lateral layer	0.980	NS
Treatment*Depth*Lateral layer	0.999	NS

In the P-stained soil, there were no significant differences between the lateral layers but the NH_4 content decreased with depth (Figure 4.7; Table 4.9). The same trend was observed for NH_4 content in P-non stained but the magnitude was lower (Figure 4.8; Table 4.10). In the P-non stained soil, which was collected from the identical layers of soil, there were no significant differences between layers. Effectively, these soil samples were outside the influence of applied effluent (Figure 4.7; Table 4.9). Similar trends were observed for the D-stained (Figure 4.8; Table 4.10) and D-non stained (Figure 4.9; Table 4.11) soil.

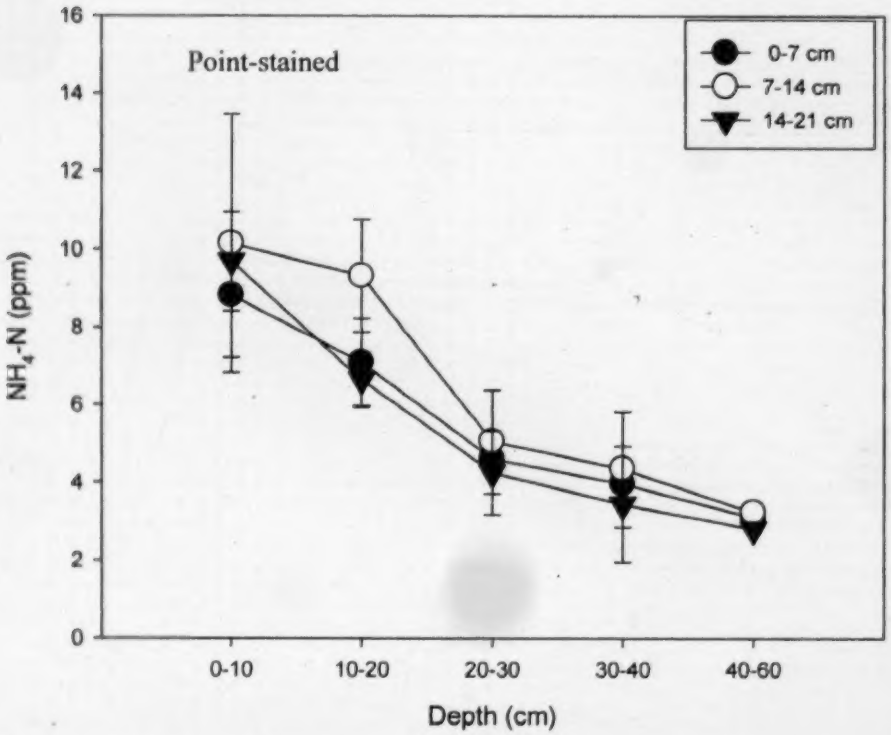

Figure 4.7. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.9. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, NH_4-N (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	8.9 ab	10.2 a	9.7 ab
10-20	7.1 abc	9.3 ab	6.7 bcd
20-30	4.6 cde	5.1 cde	4.3 cde
30-40	4.0 cde	4.4 cde	3.5 de
40-60	3.1 e	3.3 e	2.8 e

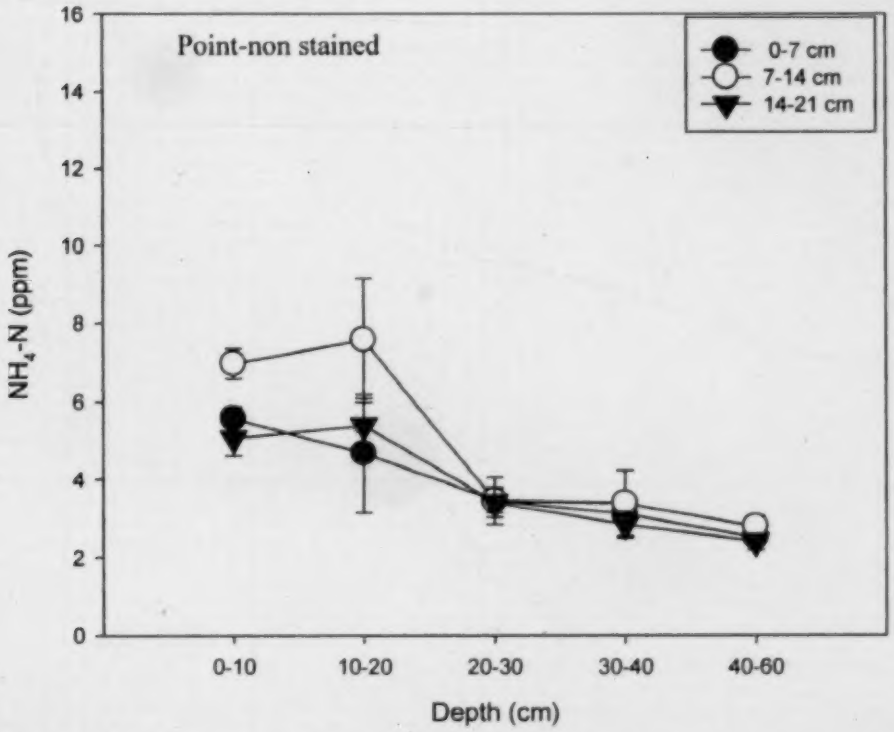

Figure 4.8. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.10. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

	Non stained soil, NH_4-N (ppm)		
Depth cm	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	5.6 abc	7.0 ab	5.1 bcde
10-20	4.7 cdef	7.5 a	5.4 bcd
20-30	3.5 defg	3.5 defg	3.4 defg
30-40	3.1 efg	3.4 defg	2.9 fg
40-60	2.5 g	2.8 fg	2.4 g

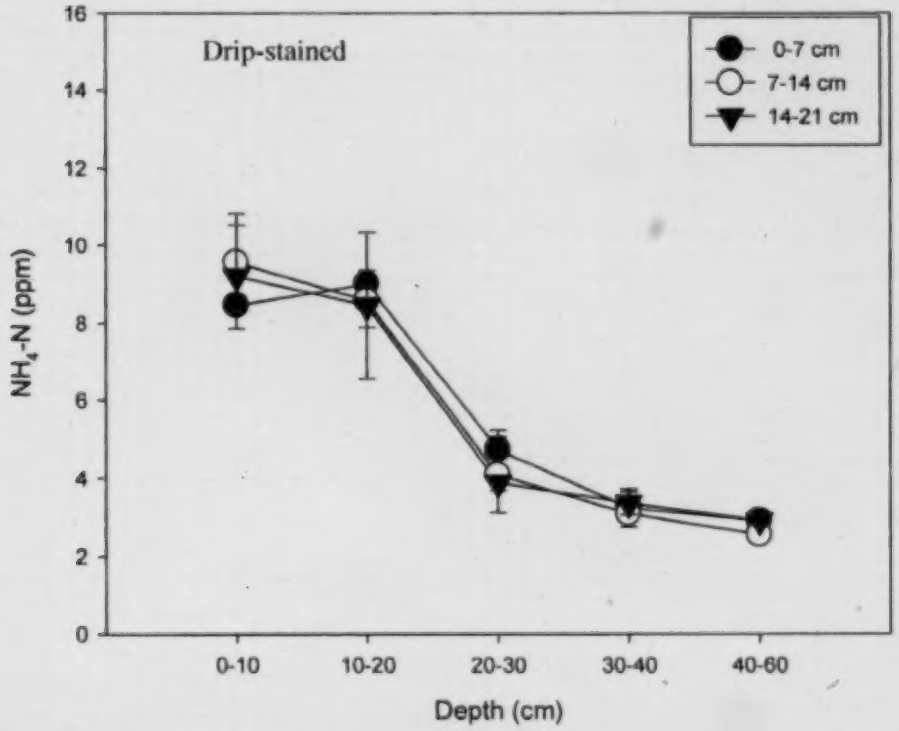

Figure 4.9. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.11. NH_4-N in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, NH_4-N (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	8.5 a	9.6 a	9.2 a
10-20	9.0 a	8.6 a	8.5 a
20-30	4.8 b	4.1 b	3.9 b
30-40	3.3 b	3.1 b	3.3 b
40-60	2.9 b	2.6 b	2.9 b

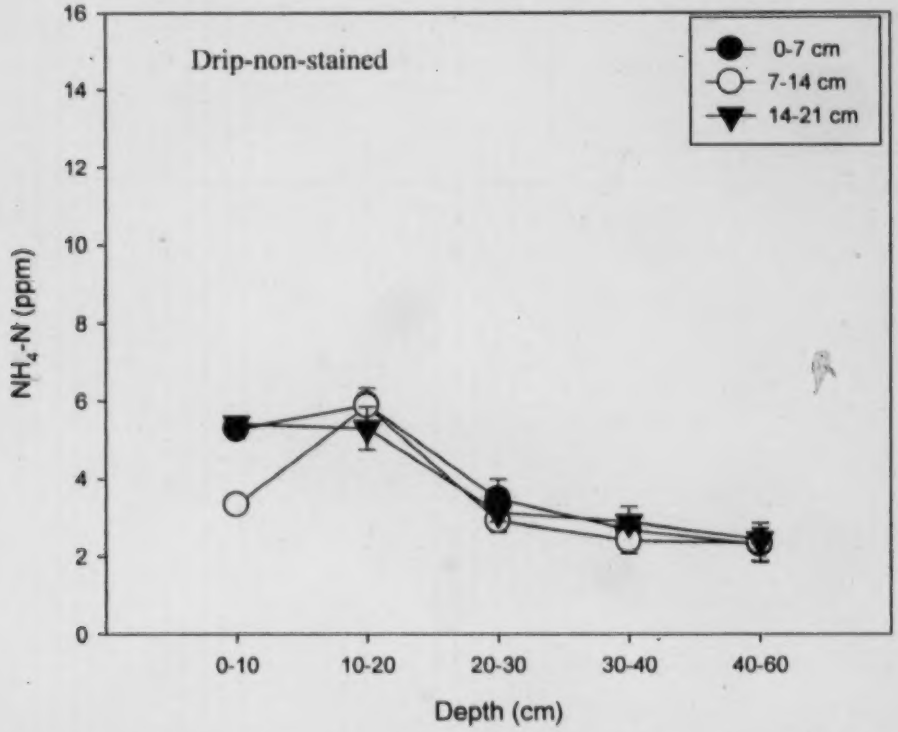

Figure 4.10. NH_4-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.12. NH_4-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Non stained soil, NH_4-N (ppm) 1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	5.3 a	3.3 a	5.4 a
10-20	5.9 a	5.9 a	5.3 a
20-30	3.5 b	2.9 bc	3.1 bc
30-40	2.7 bc	2.4 c	2.9 bc
40-60	2.3 c	2.4 c	2.4 c

4.4.4 NO_3-N content:

ANOVA showed that all main effects and interactions were not significant (Table 4.13). This was also reflected in Figure 4.11 to 4.14 and Table 4.14 to 4.17.

Table 4.13. Probability of F values from analysis of variance of NO_3-N in the field experiment (Treatment: Point and Drip; Stain: stained and non-stained soil; 3 lateral soil layer; 5 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.179	NS
Stain	0.057	NS
Treatment* Stain	0.220	NS
Depth	0.341	NS
Depth*Treatment	0.464	NS
Depth* Stain	0.494	NS
Treatment* Stain *Depth	0.454	NS
Lateral layer	0.435	NS
Treatment*Lateral layer	0.396	NS
Stain *Lateral layer	0.409	NS
Treatment* Stain *Lateral layer	0.437	NS
Depth*Lateral layer	0.431	NS
Treatment*Depth*Lateral layer	0.544	NS
Depth* Stain *Lateral layer	0.433	NS
Treatment*Depth*Lateral layer	0462	NS

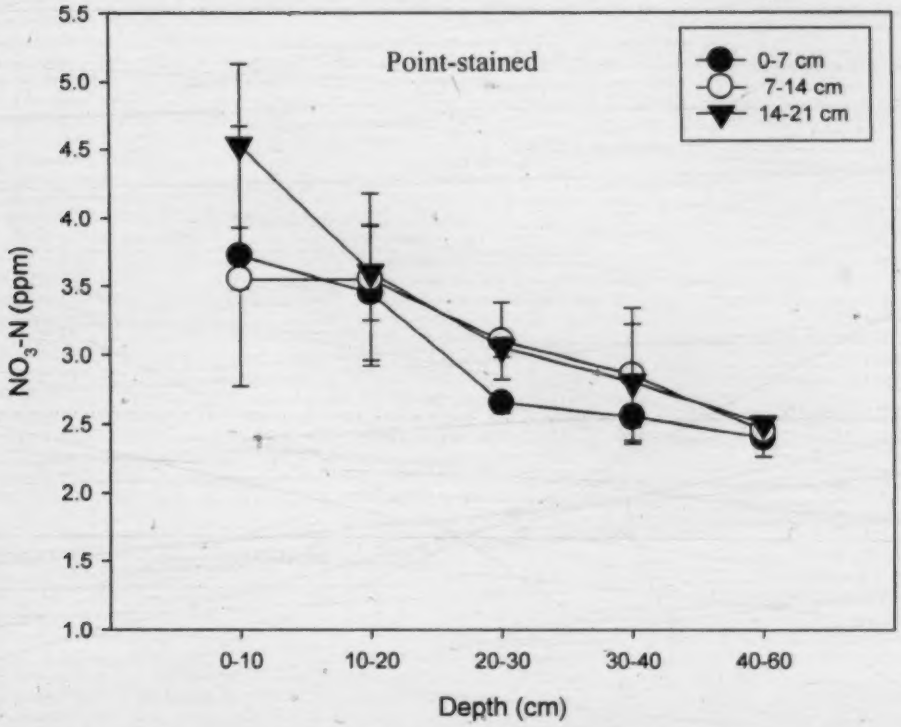

Figure 4.11. NO_3-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.14. NO_3-N in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, NO_3-N (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	3.7 ab	3.6 bc	4.5 a
10-20	3.5bcd	3.6 bc	3.6 ab
20-30	2.7 cde	3.1 bcde	3.1 bcde
30-40	2.6 cde	2.8 bcde	2.8 bcde
40-60	2.4 e	2.5 de	2.5 de

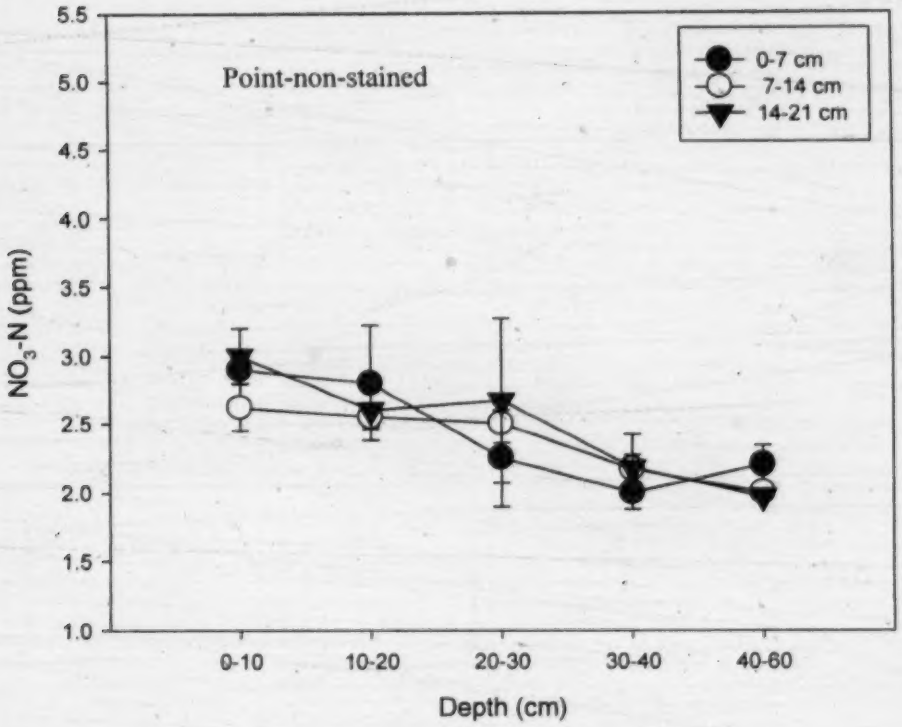

Figure 4.12. NO_3-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.15. NO_3-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Non stained soil, NO_3-N (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	2.9 a	2.6 abc	3.0 a
10-20	2.8 ab	2.6 abcd	2.6 abc
20-30	2.3 bcde	2.5 abcde	2.7 abc
30-40	2.0 de	2.2 cde	2.2 cde
40-60	2.2 cde	2.0 de	1.9±0.03 e

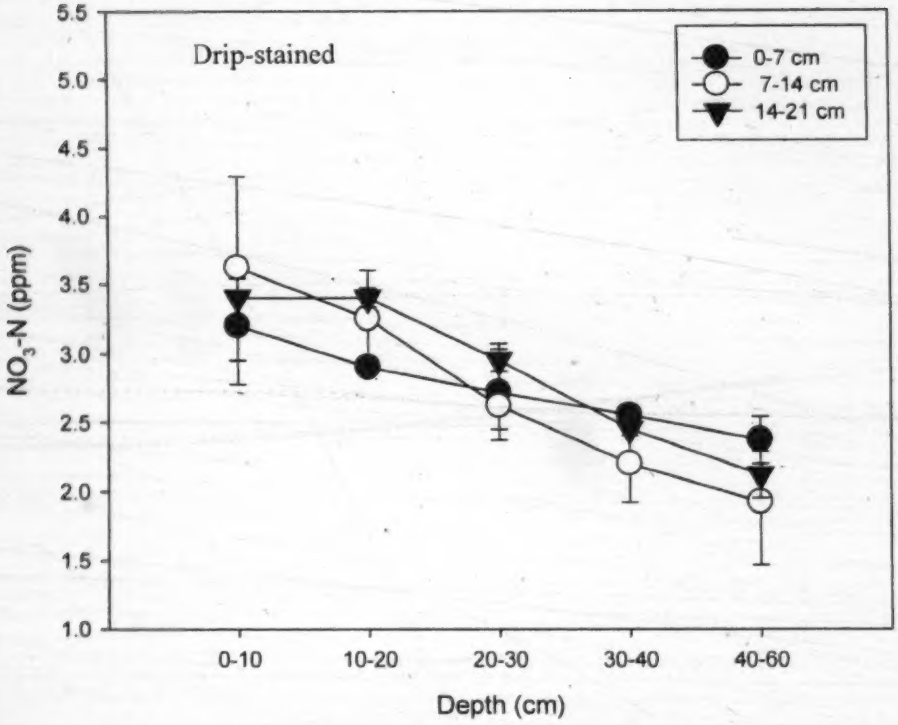

Figure 4.13. NO_3-N in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.16. NO_3-N in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

	Stained soil, NO_3-N (ppm)		
Depth cm	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	3.2 abcd	3.6 a	3.4 ab
10-20	2.9 bcde	3.3 abc	3.4 ab
20-30	2.7 cdef	2.6 cdef	2.9 bcde
30-40	2.6 defg	2.2 fg	2.4 efg
40-60	2.4 efg	1.9 g	2.1 fg

Table 4.17. NO_3-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Non stained soil, NO_3-N (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	2.8 abc	2.9 ab	3.0 a
10-20	2.4 cd	2.7 abc	2.7 abc
20-30	2.5 bcd	2.4 cd	2.6 abcd
30-40	2.1 def	1.8 f	2.3 cde
40-60	1.9 ef	1.8 f	1.9 ef

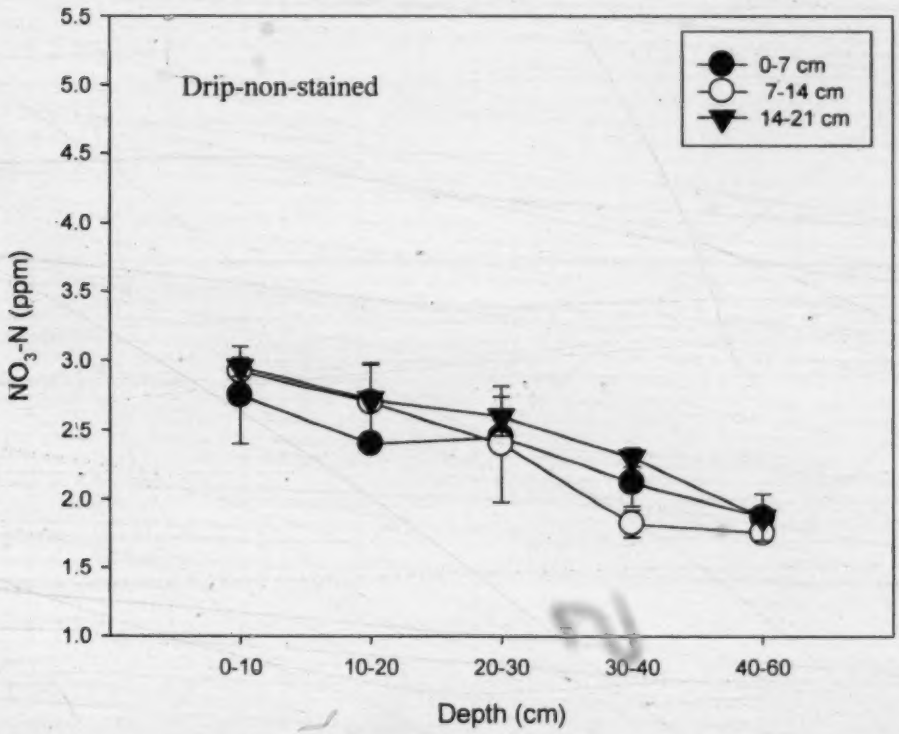

Figure 4.14. NO_3-N in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

4.4.5 Kelowna-P content:

ANOVA showed that the stain, depth and treatment*lateral layer were significant for the Kelowna-P content of the soil (Table 4.18)

Table 4.18. Probability of F values from analysis of variance of Kelowna-P in the field experiment (Treatment: Point and Drip; Stain: stained and non stained soil; 3 lateral soil layer; 5 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.613	NS
Stain	0.012	Significant
Treatment* Stain	0.578	NS
Depth	<0.001	Significant
Depth*Treatment	0.959	NS
Depth* Stain	0.155	NS
Treatment*Stain*Depth	0.390	NS
Lateral Layer	0.512	NS
Treatment*Lateral Layer	0.046	Significant
Stain *Lateral Layer	0.446	NS
Treatment* Stain *Lateral Layer	0.084	NS
Depth*Lateral Layer	0.715	NS
Treatment*Depth*Lateral Layer	0.123	NS
Depth* Stain *Lateral Layer	0.409	NS
Treatment*Depth*Lateral Layer	0.665	NS

In the P-stained soil, there were no significant differences between the lateral layers (except for the 0-10 cm layer) but the Kelowna-P content decreased with depth (Figure 4.15; Table 4.19). The same trend was observed for Kelowna-P content in P-non stained but the magnitude was lower (Figure 4.16; Table 4.20). Similar trends were observed for the D-stained (Figure 4.17; Table 4.21) and D-non stained (Figure 4.18; Table 4.22).

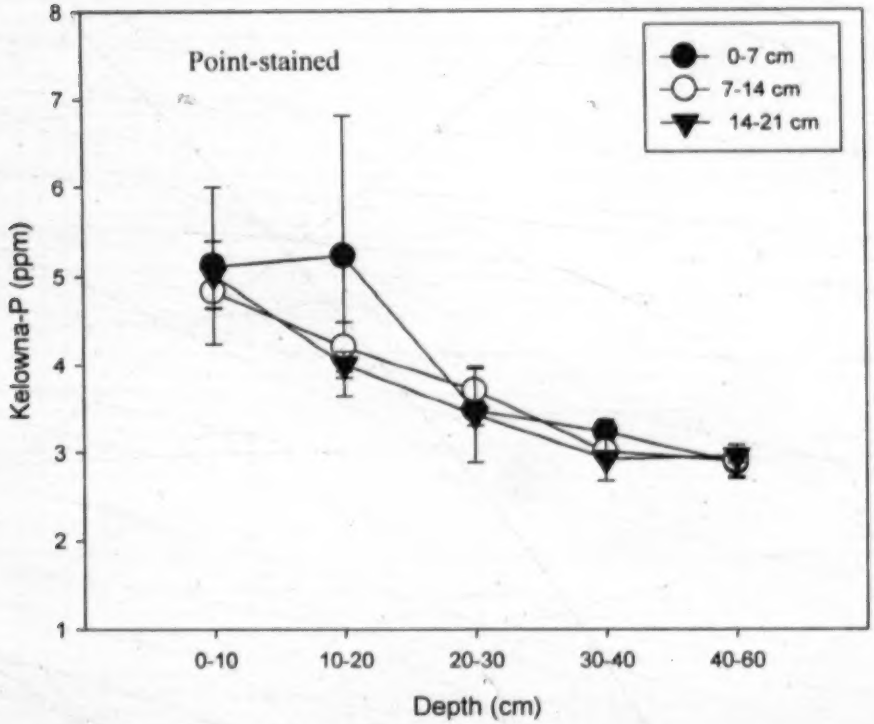

Figure 4.15. The content of Kelowna-P in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.19. The content of Kelowna-P in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Kelowna-P (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layer (14-21 cm)
0-10	**5.1 a**	**4.8 b**	**5.0 a**
10-20	5.2 a	4.2 abc	4.0 abcd
20-30	3.4 cd	3.7 bcd	3.4 cd
30-40	3.2 cd	3.0 cd	2.9 d
40-60	2.8 d	2.9 d	2.9 cd

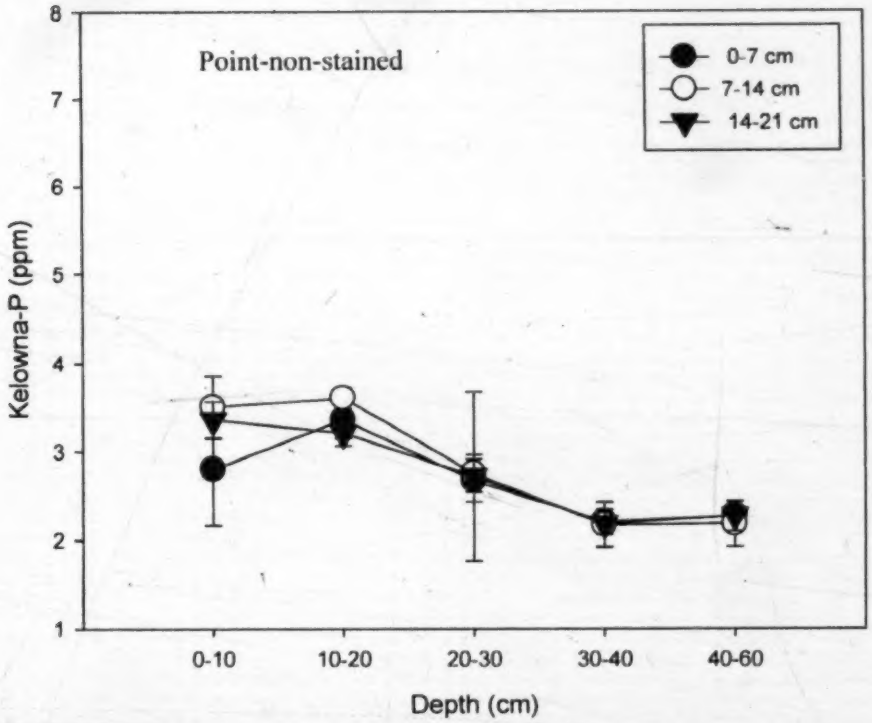

Figure 4.16. The content of Kelowna-P in five different depths and three different lateral layers of the non stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.20. The content of Kelowna-P in five different depths and three different lateral layers of the non stained soil in point application system.

Depth cm	Non stained soil, Kelowna-P (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	2.8 abc	3.5 ab	3.3 ab
10-20	3.4 ab	3.6 a	3.2 ab
20-30	2.6 bc	2.7 abc	2.7 bc
30-40	2.2 c	2.1 c	2.1 c
40-60	2.2 c	2.1 c	2.2 c

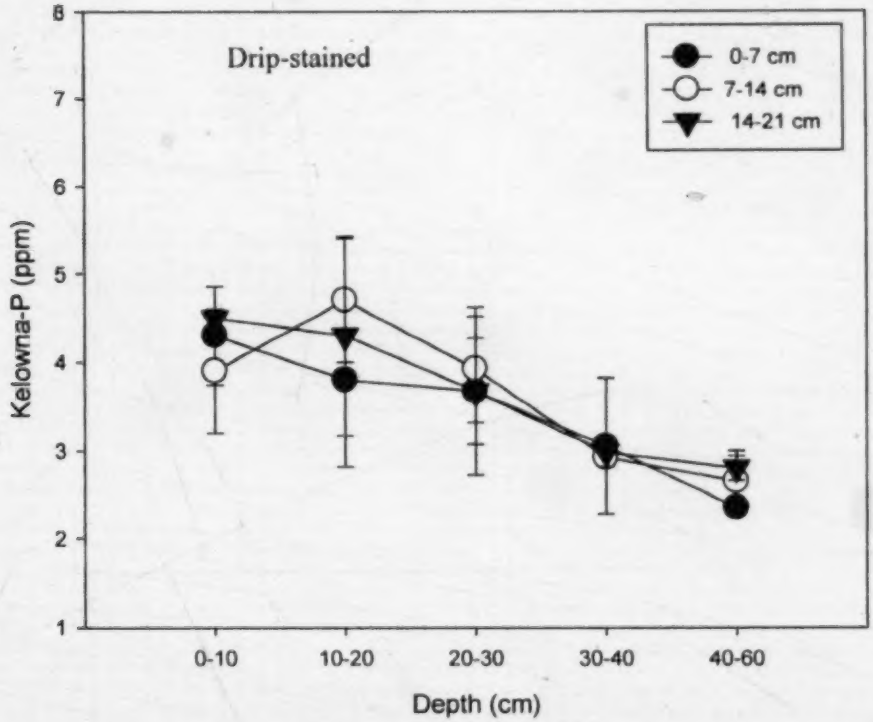

Figure 4.17. The content of Kelowna-P in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.21. The content of Kelowna-P in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Kelowna-P (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	4.3 abc	3.9 abcd	4.5 ab
10-20	3.8 abcd	4.7 a	4.3 abc
20-30	3.7 abcd	3.9 abcd	3.7 abcd
30-40	3.1 bcd	2.9 bcd	2.9 bcd
40-60	2.3 d	2.7 d	2.8 cd

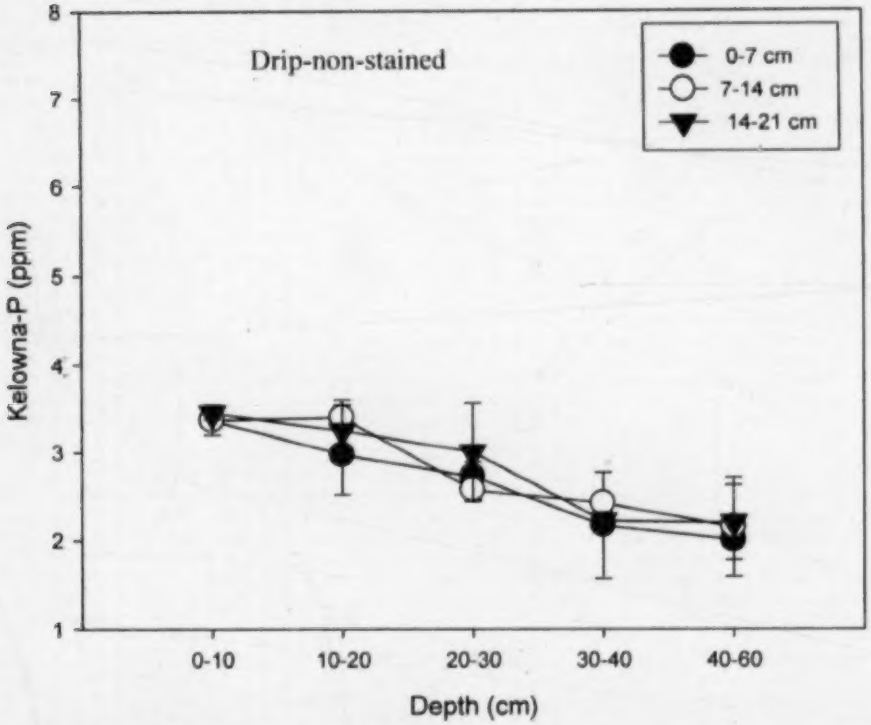

Figure 4.18. The content of Kelowna-P in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.22. The content of Kelowna-P in five different depths and three different lateral layers of the non stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Non stained soil, Kelowna-P (ppm)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	3.4 a	3.4 a	3.5 a
10-20	3.0 abcd	3.4 a	3.3 ab
20-30	2.7 abcd	2.5 bcd	3.0 abc
30-40	2.2 e	2.4 cde	2.2 cde
40-60	2.0 e	2.2 e	2.2 de

4.4.6 Fecal coliforms:

There were no fecal coliforms in unstained soil, therefore the ANOVA table is shorter (Table 4.23). Soil fecal coliforms differ significantly among the treatments, depths, lateral layer, and other interactions (Table 4.23).

Table 4.23. Probability of F values from analysis of variance of fecal coliforms in stained soil from the field experiment (Treatment: Point and Drip; Stain: stained and non stained soil; 3 lateral soil layer; 5 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.0001	Significant
Depth	<0.0001	Significant
Depth*Treatment	0.0442	Significant
Lateral layer	<0.0001	Significant
Treatment*Lateral layer	0.2226	NS
Depth*Lateral layer	<0.0001	Significant
Treatment*Depth*lateral layer	0.8387	NS

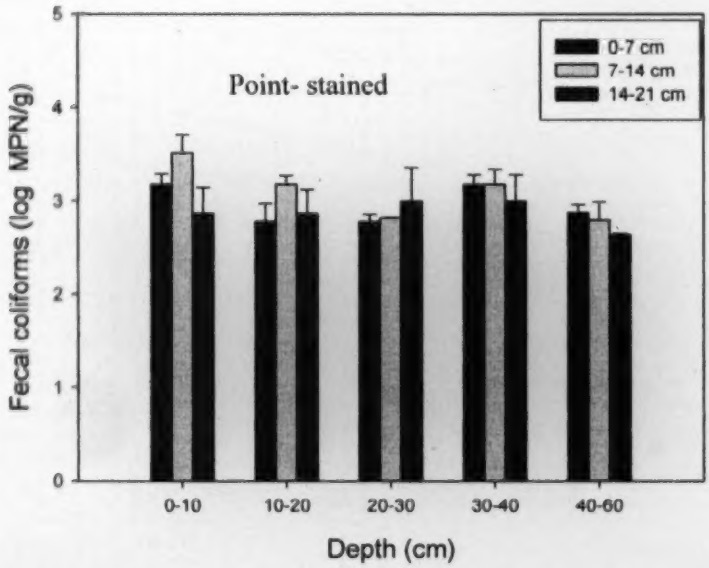

Figure 4.19. Fecal coliforms in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Error bar shows standard deviation (±).

Table 4.24. Number of Fecal coliforms (log MPN/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Fecal coliforms (log MPN/g)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	3.2 ab	3.5 a	2.9 ab
10-20	2.8 ab	3.2 ab	2.9 ab
20-30	2.8 ab	2.8 ab	3.0 ab
30-40	3.2 ab	3.2 ab	3.0 ab
40-60	2.9 ab	2.8ab	2.7 ab

There were no significant difference in the fecal coliform population between layers and with depth in the P-stained soil samples, however the log MPN/g were in the order of 4.6 to 5.5 (Figure 4.19; Table 4.24).

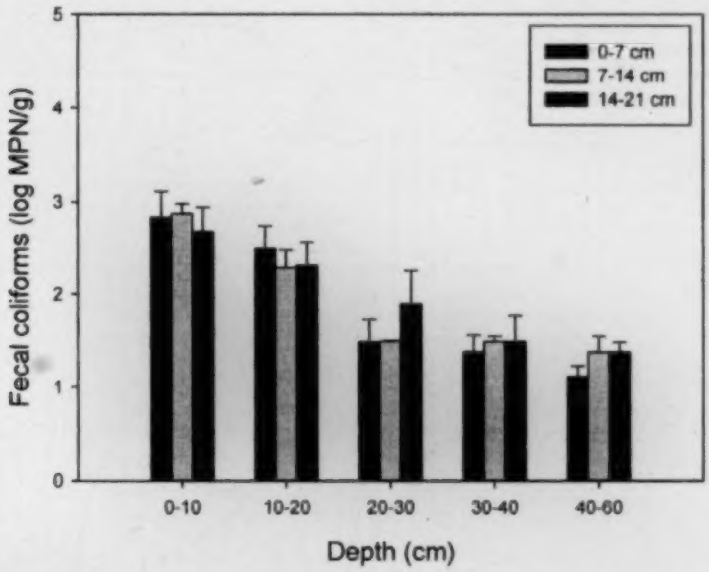

Figure 4.20. Number of Fecal coliforms (log MPN/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Error bar shows standard deviation (±).

Table 4.25. Number of Fecal coliforms (log MPN/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Fecal coliforms (log MPN/g)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	**2.8 a**	**2.9 a**	**2.7 ab**
10-20	**2.5 ab**	**2.3 abc**	**2.3 abc**
20-30	1.5 cd	1.5 abc	1.9 bcd
30-40	1.4 d	1.5 cd	1.5 cd
40-60	1.1e	1.4 d	1.4 d

In D-stained soil, fecal coliforms were significantly higher in the 0-10 and 10-20 cm depths and also decreased significantly with depth (Figure 4.20; Table 4.25).

4.4.6 Somatic Coliphages:

The depth and depth*treatment effects were significant for somatic coliphages (Table 4.26).

Table 4.26. Probability of F values from analysis of variance of fecal coliforms in stained soil from the field experiment (Treatment: Point and Drip; 3 lateral soil layers; 5 vertical depths).

Source	Pr(>F)	Significance at 95% confidence level
Treatment	0.563	Non significant
Depth	<0.001	Significant
Depth*Treatment	0.003	Significant
Lateral layer	<0.386	Non significant
Treatment*Lateral layer	0.083	Non significant
Depth*Lateral layer	<0.122	Non significant
Treatment*Depth*lateral layer	0.725	Non significant

In the P-stained soil, the somatic coliphages were distributed in all layers to a depth of 60 cm (Figure 4.21; Table 4.27) however in the D-stained soil, they were present in all lateral layers but decreased significantly with depth (Figure 4.22; Table 4.28). Attenuation of water also attenuated somatic coliphages.

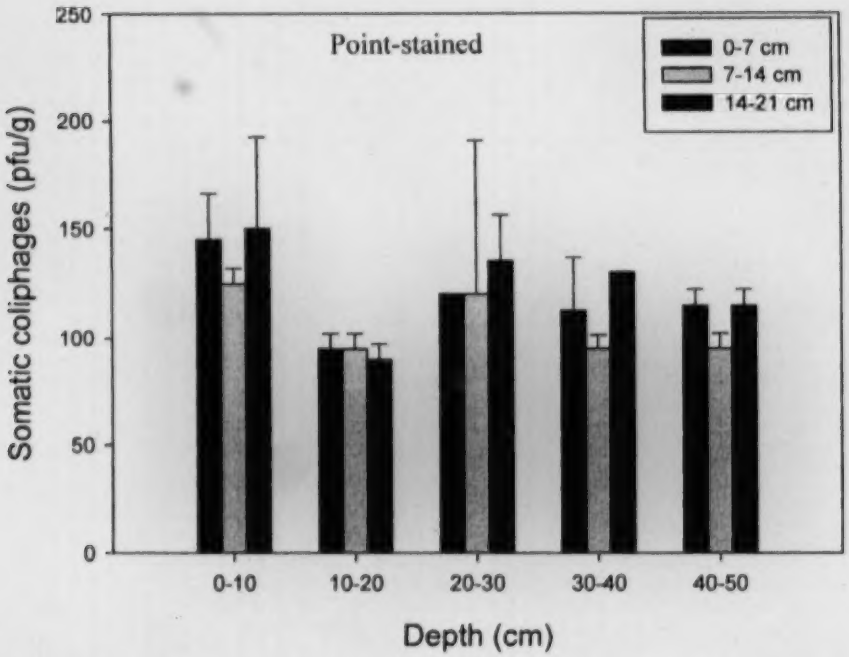

Figure 4.21. Number of somatic coliphages (pfu/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the point application. Error bar shows standard deviation (±).

Table 4.27. Number of somatic coliphages (pfu/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the point application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Somatic coliphages (pfu/g)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	145.0 abc	125.0 abcd	150.0 ab
10-20	95.0 bcd	85.0 d	90.0 cd
20-30	120.0 abcd	160. 0a	135.0 abcd
30-40	112.0 abcd	94.5 bcd	130.0 abcd
40-60	115.0 abcd	95.0 bcd	115.0 abcd

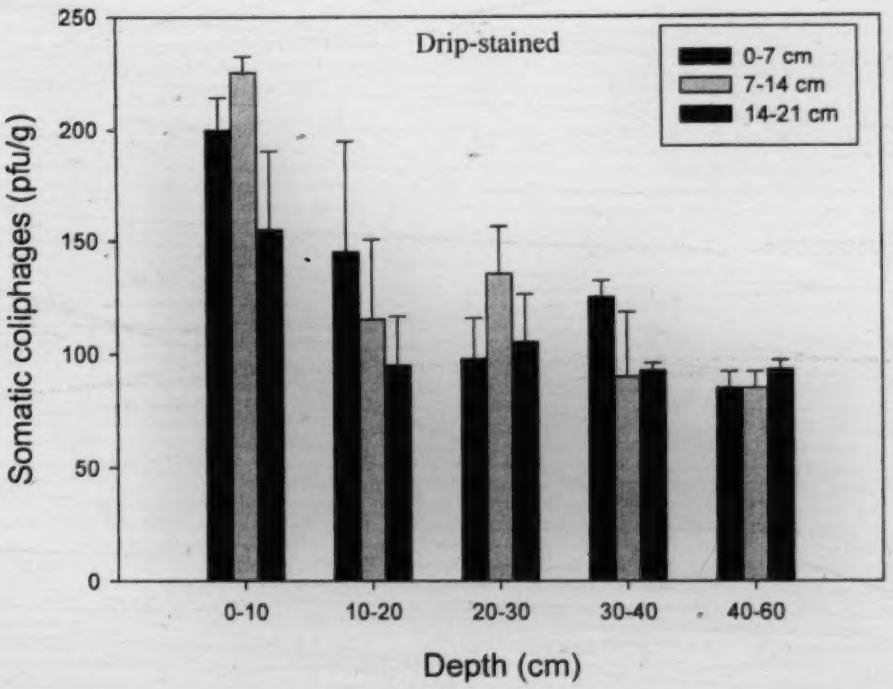

Figure 4.22. Number of somatic coliphages (pfu/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application. Error bar shows standard deviation (±).

Table 4.28. Number of somatic coliphages (pfu/g) in five different depths and three different lateral layers of the stained soil receiving effluent from the drip application method. Treatments not followed by the same alphabetical letters are statistically significant at P=0.05 (Duncan test).

Depth cm	Stained soil, Somatic coliphages (pfu/g)		
	1st lateral layer (0-7 cm)	2nd lateral layer (7-14 cm)	3rd lateral layers (14-21 cm)
0-10	200.0 ab	225.0 a	155.0 bc
10-20	145.0 cd	115.0 cde	95.0 de
20-30	97.5 de	135.0 cde	105.0 cde
30-40	125.0 cde	90.0 de	92.0 de
40-60	85.0 e	85.0 e	93.0 de

4.6 Discussion:

Water distribution pattern plays an important role for the movement of nutrients and organisms through the soil. In the present study, drip dispersal method was compared to conventional point application method with the assumptions that the drip method will allow effluent dispersal in an even and controlled manner. Moreover, it will cause much more lateral movement of wastewater by capillary action rather than by gravitation flow. The goal of this technique is to allow soil more time to purify pathogens and store nutrients in the soil.

4.6.1 Key Discoveries

Here are the key results from this field experiment:

1. The Brilliant Blue food dye used to trace the distribution of wastewater in an Eluviated Black Chernozem resulted in a very clear, visible boundary for the stained soil. The protocol for sampling separated the stained soil from the non-stained soil permitted the comparison of the effluent impacted area from soils not impacted by effluent in terms of gravimetric moisture content, nutrient content (ammonium, nitrate and Kelowna-P), fecal coliforms and somatic coliphages.
2. Fecal coliforms were only found in the effluent impacted soil. After 15 daily doses of the diluted primary effluent, the distribution of fecal coliforms by the point method resulted in an almost even distribution around the point of application. The dimensions of the affected area resembled a cylinder with a radius of 21 cm and a depth of 60 cm. In the drip application, there was more even and controlled movement of water and the moisture content in the 10-20 cm depth was higher than in the 0-10 cm depth. In the effluent impacted soil by the drip method, fecal coliforms were significantly higher in the 0-10 and 10-20 cm depth and also decreased significantly with depth. These data showed that the drip method provided a better treatment of fecal coliforms which were added to the soil over a period of 15 days.
3. Somatic coliphages were only found in the stained soil. They were almost evenly distributed in the stained soil to a depth of 60 cm which received DPE by the point application method. In contrast, number of somatic coliphages which received DPE

by the drip application method was distributed to a depth of 60 cm but their numbers decreased significantly with depth. These data show that there was better treatment of somatic coliphages in the drip application method.

4. On average, the infiltrated stained wastewater was transported vertically to an average depth of 49 cm laterally to 31.5 cm in the point application. In contrast, the infiltrated stained wastewater was transported vertically to an average depth of 44 cm laterally to 28.5 cm in the drip application. Therefore, in the point application method, the wastewater spread more from the point of application and went to a deeper depth. There was uneven distribution of the effluent in the point application method compared to the drip application method.
5. The nutrient content (NH_4-N, NO_3-N and Kelowna-P) of the effluent impacted soils from both methods of application were higher than non stained soil. Nutrient content decreased with depth in this soil.

4.7 Conclusion

In Chapter 3, important factors that should be considered for better designs are: loading rates based on soil type, orifice spacing, application methods, size and shape of chambers, and direction of orifices on the laterals. This field experiment has partially addressed the issues of application methods at one loading rate based on soil without restricting layers and has yielded results which showed that the drip application method was better than point application method. However, this statement has to be interpreted with great caution because the experiment was a simulation of applying diluted primary effluent to a soil for only 15 days. A more rigorous research program is needed to address many complex issues of soil-based wastewater dispersal systems.

4.8 References:

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Chapter 5

Conclusions and Implications

5.1 Conclusions

This thesis focus on two major points: (1) point loading of effluent in soil-based dispersal fields; and (2) methods of applying wastewater to solve the point loading problem. In at-grade onsite wastewater treatment systems, effluent is applied directly on to the soil to ensure its purification by soil and their safe release into the groundwater. In the present study, experiments were conducted with wastewater to compare the performance of point and drip application methods under laboratory and field conditions. The major findings from the soil column study are presented below.

The following conclusions were drawn from the column experiment (Chapters 2 &3):

1. Transient saturated flow obtained in case of point application system due to intermittent ponding.
2. Transient unsaturated flow obtained due to intermittent dripping.
3. Water content in the lower depth (65 cm) was higher compared to other two depths (15 cm and 35 cm).
4. The water content was higher in soil received water in point application system in both horizontal depth as well as vertical depths than soil in columns received effluent in drip application system which might causes difference in bacterial transport.
5. NH_4-N, NO_3-N, soluble-P and somatic coliphages were detected in leachate samples throughout the experimental period but fecal coliforms were not detected in the leachates.
6. NH_4-N, NO_3-N and Kelowna-P in soil decreased significantly ($P=<0.05$) with depth and from central core to second torus in both application methods.
7. During the 60 to 90 day period, there was movement of fecal coliforms in soil columns from 35-55 cm to 55-75 cm depth in the point method and from 20-35 to 35-55 cm depth in the drip method.
8. Fecal coliforms and somatic coliphages decreased significantly ($P=<0.05$) from central core to second torus in both application systems.

Here are the key results from this field experiment (Chapter 4):

1. The Brilliant Blue food dye used to trace the distribution of wastewater in an Eluviated Black Chernozem resulted in a very clear, visible boundary for the stained soil. The protocol for sampling separated the stained soil from the non-stained soil permitted the comparison of the effluent impacted area from soils not impacted by effluent in terms of gravimetric moisture content, nutrient content (ammonium, nitrate and Kelowna-P), fecal coliforms and somatic coliphages.
2. Fecal coliforms were only found in the effluent impacted soil. After 15 daily doses of the diluted primary effluent, the distribution of fecal coliforms by the point method resulted in an almost even distribution around the point of application. The dimensions of the affected area resembled a cylinder with a radius of 21 cm and a depth of 60 cm. In the drip application, there was more even and controlled movement of water and the moisture content in the 10-20 cm depth was higher than in the 0-10 cm depth. In the effluent impacted soil by the drip method, fecal coliforms were significantly higher in the 0-10 and 10-20 cm depth and also decreased significantly with depth. These data showed that the drip method provided a better treatment of fecal coliforms which were added to the soil over a period of 15 days.
3. Somatic coliphages were only found in the stained soil. They were almost evenly distributed in the stained soil to a depth of 60 cm which received DPE by the point application method. In contrast, number of somatic coliphages which received DPE by the drip application method were distributed to a depth of 60 cm but their numbers decreased significantly with depth. These data show that there was better treatment of somatic coliphages in the drip application method.
4. On average, the infiltrated stained wastewater was transported vertically to an average depth of 49 cm laterally to 31.5 cm in the point application. In contrast, the infiltrated stained wastewater was transported vertically to an average depth of 44 cm laterally to 28.5 cm in the drip application. Therefore, in the point application method, the wastewater spread more from the point of application and went to a deeper depth. There was uneven distribution of the effluent in the point application method compared to the drip application method.

5. The nutrient content (NH_4-N, NO_3-N and Kelowna-P) of the effluent impacted soils from both methods of application were higher than non stained soil. Nutrient content decreased with depth in this soil.

5.2 Implications for the Onsite Industry and Development of New Guidelines

Important factors that should be considered for better designs are: application methods, loading rates based on soil type, orifice spacing, size and shape of chambers, and direction of orifices on the laterals. This research project has partially addressed the issues of application methods on two soils under laboratory and field conditions and has yielded results which showed that the drip application method was better than point application method. It is very clear that point loading causes saturated flow and enhances the movement of fecal coliforms, somatic coliphages and nutrients such as NH_4-N, NO_3-N, and Kelowna-P. If the loading rates are reduced and if the method of applying effluents is improved, then there are very good chances of increasing treatment effectiveness and reducing environmental pollution. The results of this thesis also need to be tested on fully functional, existing onsite systems.